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THE EFFECT OF GALACTOSE ON THE GROWTH OF CERTAIN FUNGI

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(WITH 10 FIGURES)

LITERATURE REVIEW

The toxic effect of galactose on green plants was observed first by Knudson (**7**) in his experiments on vetch (*Vicia villosa* L.) and Canada field pea (*Pisum sativum* L.). He found that plants when grown in two per cent galactose media, very early in their development showed browning, discoloration, and marked injury—the injury being manifested by a killing of the roots and accompanied by a reduction in the growth of the tops. He showed, furthermore, that whereas galactose acted deleteriously toward the roots of the Canada field pea, the sugars, glucose and sucrose, acted beneficially when compared with the check cultures of green plants grown without sugar. The manner in which injury is caused by the galactose was not determined by Knudson. He suggested, however, the possibility that the oxidation products of galactose are the injurious agents causing the toxic effects on green plants.

In a later paper Knudson (**8**) discovered that mannose sugar behaved in a manner similar to that of galactose, causing discoloration, injury and retardation of root growth in the presence of mannose at a concentration of 0.025 mol. He found also that the toxic effects of galactose and of mannose sugars were prevented in the presence of an equal concentration of glucose or sucrose.

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Knudson failed, however, to account for the manner in which these harmful effects were caused other than by his original suggestion in regard to the possible toxic nature of the oxidation products of galactose. In explanation of the antagonism shown to exist between galactose and glucose, in which the roots of green plants showed no injurious effects when grown on media containing these sugars combined in equal concentrations, he offered the suggestion that there existed a selective phenomenon in the plant preventing the absorption of galactose in the presence of glucose.

In a series of experiments on the pea (*Pisum sativum* L.) Heinonen (**4**), elaborating on the experiments of Knudson, found in the main results similar to those reported by Knudson. She discovered that the affected roots showed bulbous enlargements toward the tips as well as marked discoloration followed by eventual death of the roots. In addition she found that green plants grown in media containing a mixture of galactose and glucose exhibited antagonism only so long as the glucose was present in the culture media. When growth of the green plants was extended over a period of time sufficiently long to exhaust the glucose contained within the culture media, then the galactose present showed its toxic effects through responses of the roots in exhibiting discolorations and other injurious features. In accordance with the suggested explanation of Knudson, she believed also that the absorption of galactose was prevented by the presence of glucose and that the toxic effects of galactose on the roots of green plants might be due to the oxidation products of galactose. She did not, however, offer any experimental proof to substantiate this belief.

Investigating the effect of galactose on non-green plants, Horr (**5**) used two species of fungi; namely *Aspergillus niger* van Tiegh and *Penicillum glaucum* Link. Recording his data on a quantitative dry-weight basis, Horr found for galactose, when compared with glucose, a decided decrease in the quantity of mycelium produced by the former sugar during a definite period of time when the fungi were grown on media containing two per cent concentrations. Furthermore, he found that the growth inhibiting effect of galactose was prevented in media containing a mixture of galactose and glucose. In fact an accelerated growth

response resulted in fungi raised on media containing a mixture of the sugars, galactose and glucose. In the presence of galactose Horr found that *Aspergillus niger* and *Penicillum glaucum* showed a reduction in spore germination, a retardation in the rate of mycelial growth, irregularities in the formation of hyphal threads, and a marked decrease in the quantity of mycelium produced in a definite period of time. In principle his results compared favorably with the experimental data previously recorded by Knudson and Heinonen in their experiments on green plants. He differed noticeably, however, in the tentative conclusions he drew from his experimental observations. The evidence available to Horr did not seem to warrant the interpretation offered by Knudson that the injurious effects exhibited by non-green plants were due to the toxic nature of galactose, or to its oxidation products. Horr believed rather that the retardation in growth, the decreased weight of mycelium, and other responses were due, largely, to the unavailability of the sugar galactose, which serves as a poor source of carbon for fungi, or to the slow absorption of galactose by the plant since a delayed growth rather than toxic characteristics was usually the principal plant response. He does not, however, offer any conclusive or satisfactory evidence in support of this explanation.

To some extent the work of Horr agrees with the findings recorded in an earlier paper by Matsumato (**9**) in which from direct experimental data with glucose, fructose, and galactose sugars, he inferred that all the monosaccharides were directly utilizable by various strains of *Rhizoctonia* D.C. with approximately equal availability. Matsumato found further that the same strains of *Rhizoctonia* were capable of converting sucrose into glucose and fructose as well as being able to hydrolyze starch, thus making these carbohydrates available also as sources of carbon.

Coons (**1**) studying the factors involved in the growth and reproduction of *Plenodomus fuscomaculans* Sacc. found that the sugars glucose, galactose, and sucrose served equally well as sources of carbon in stimulating and sustaining vegetative growth. He found also that the highly soluble carbohydrates, increasing the sugar concentration of the media, induced an abundant vegetative growth while the slightly soluble carbohydrates, decreasing

the sugar concentration of the media, induced reproductive development but supported only a weak vegetative growth. On the basis of the results obtained from the highly soluble and slightly soluble carbohydrates, Coons concluded that the difference in growth forms is connected with the amount of food supply available rather than with the specific nature of the sugar. It would seem to the writer that this observation, if correct, would further strengthen the position taken by Horr that the lessened availability of galactose rather than its toxic nature is instrumental in reducing the growth of non-green plants.

In view of the marked unfavorable responses of chlorophyllous plants to the carbohydrate galactose, as shown by the experiments of Knudson and Heinonen, who demonstrated the toxic nature of galactose on the roots of green plants, the work described in this paper was undertaken to discover, if possible, whether similar toxic effects could be demonstrated in the case of galactose towards non-chlorophyllous plants. Furthermore, in the event that galactose toxicity was shown to hold for non-green plants, it was the aim of the writer to attempt to further clarify the situation as to the nature of the toxic effects. The present research was completed sometime before the publication of the paper by Horr who approaches the problem from a similar angle, although he limits his investigation to two closely related species of fungi and records his data on a quantitative dry-weight basis. This article, however, as here presented, is written and discussed in the light of Horr's findings.

METHODS AND PROCEDURES

To obtain a representative selection of non-green plants, a choice of material was made from several of the subdivisions of fungi. Care was exercised, moreover, in the selection of species to secure forms with varying morphological and physiological characteristics. This was done in order to provide a large variety of forms over as broad a field of fungi as possible, and yet consistent with the adequacy of the laboratory equipment available. However, the maintenance of efficient and satisfactory manipulation of the many cultures involved in these experiments necessitated limiting the number used to six different species.

The species finally accepted were *Phytophthora Cactorum* (Leb. & Cohn) Schr. and *Saprolegnia ferax* (Graith) Thuret to represent the Phycomycetes, the species *Sclerotinia cinerea* (Bon.) Schr. and *Physalospora Cydoniae* (Berk.) Shear to represent the Ascomycetes, while *Alternaria Solani* (Ellis & Mart.) Jones and Grout and *Sclerotium Rolfsii* Sacc. were taken to represent the Fungi Imperfecti. Taken from six different genera, the six species of fungi used in this experiment were renewed from laboratory stock cultures by growth from single spore, single sclerotial initial or hyphal tip—the pure cultures being acquired through the dilution method. After purification of the fungi, they were grown for several generations on ordinary potato media to confirm and check their approximation to type cultures. The fungi were then grown on the basic experimental media before being used in this experiment.

The three basic media chosen as comparative substrata for this research were those of Czapek, Waksman and Sabouraud. The principal ingredients in Czapek's media, in Waksman's and Sabouraud's media are given in grams (Table I).

TABLE I

INGREDIENTS IN GRAMS

Czapek	Waksman	Sabouraud
2 $NaNO_3$	5 Peptone	10 Peptone
1 KH_2PO_4	1 KH_2PO_4	15 Agar
.5 KCl	.5 $MgSO_4$ 7 H_2O	2% (Carbohydrates)
.5 $MgSO_4$ 7 H_2O	15 Agar	Litre Dist. Water
.01 $FeSO_4$	2% (Carbohydrates)	
15 Agar	Litre Dist. Water	
2% (Carbohydrates)		
Litre Dist. Water		

Four carbohydrates were used, namely glucose, galactose, sucrose and starch. These four carbohydrates were each separately substituted in a two per cent concentration in all three of the basic media, namely Czapek, Waksman, and Sabouraud. The supposedly toxic galactose was observed in contrast to the non-toxic carbohydrates, glucose, sucrose and starch. The media thus combined for these experiments then may be further simplified by an examination of the arrangement given in Table II. The abbreviated symbols, introduced here (Table II), will be main-

tained throughout the experimental and discussional portions of this paper.

TABLE II

SYMBOLS FOR CARBOHYDRATE MEDIA

	Czapek	Waksman	Sabouraud
Glucose	Cz–gl	Wk–gl	Sa-gl
Galactose	Cz–ga	Wk–ga	Sa–ga
Sucrose	Cz–su	Wk–su	Sa–su
Starch	Cz–st	Wk–st	Sa–st

All ingredients used in these media, whether carbohydrate or mineral in nature, were introduced as chemically pure substances. The reaction of the media in all instances was adjusted to the neutral point and the media were solidified with one and one-half per cent agar. The media were sterilized in an autoclave for 20 minutes at 15 lbs. pressure, and the sugars were added after sterilization by filtration.

Each fungus used in this experiment was grown in triplicate sets on all twelve combinations of media established (Table II). In the beginning the main difficulty was to determine a procedure to grow the fungi under uniform conditions of temperature and moisture, and to record the data at approximately uniform intervals of time. The fungi were developed in large-sized petri dishes containing a uniformly thick layer of culture media. Records were made daily and graphs were constructed from the average of readings from each triplicate set of uniform growth. The fungi were grown in the dark in an incubator at a constant moisture content and a temperature of 23° C. In all cases the data observed were recorded as a linear measurement of the vegetative growth rate, estimated on the basis of the diameter of the fungous surface-mat. The readings were taken by transmitted light with the low magnification objective of a compound microscope.

In all inoculations made during the course of the experiments, the cultures were planted by means of a nichrome loop so fashioned as to transfer always a uniformly-sized disk of the fungous mat. For the sake of conciseness the data here assembled are presented largely in the form of composite graphs.

EXPERIMENTATION AND DATA

The experimental data is recorded under three series of experiments. The first series of experiments, covering the tests on Czapek's media, is given graphically and in detail; the second

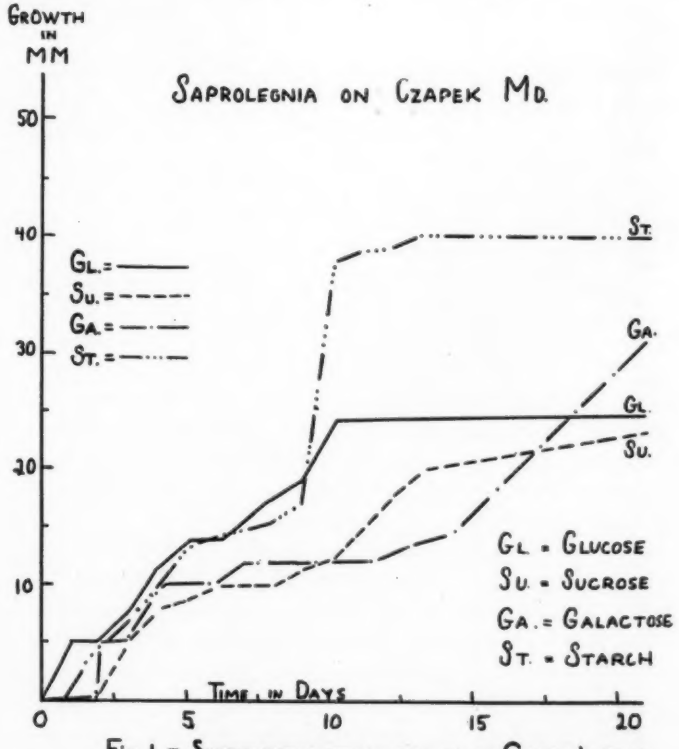

FIG. 1 - SAPROLEGNIA FERAX GROWN ON CZAPEK'S MEDIA

series, covering the work on Waksman's media, is given graphically but by only one example; while the third series of experiments, covering the work on Sabouraud's media, is given in brief form similar to that of the second series of experiments.

The graph in figure 1 shows the vegetative growth response of the fungus *Saprolegnia ferax* on Czapek's media in the presence of the four carbohydrates indicated by lines on the graph and by

symbols; namely, Cz-gl, Cz-ga, Cz-su, Cz-st (Table II). The growth rate of *Saprolegnia ferax,* taken as a linear measurement of the diameter of the surface fungous-mat, is recorded in milli-

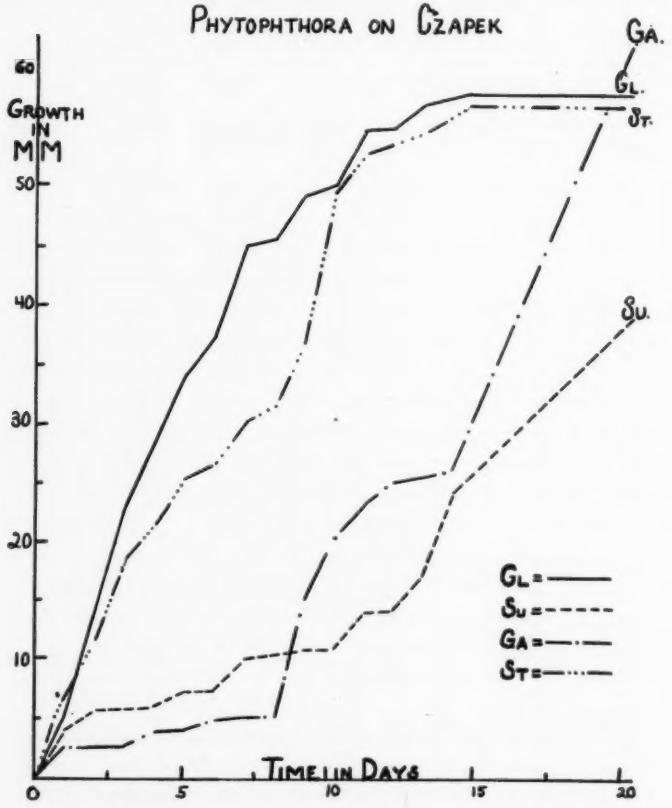

FIG 2 - PHYTOPHTHORA CACTORUM GROWN ON CZAPEK'S MEDIA

meters over equal periods of time in days. Under the conditions of this experiment, growth is decidedly lessened in the presence of galactose compared with the rate of growth in the presence of glucose. In this instance, with *Saprolegnia ferax* the growth rate is also relatively low on sucrose.

In figure 2 the experimental conditions are the same as those standardized for figure 1. The vegetative growth response of *Phytophthora Cactorum* on Czapek's media for the four carbohydrates under consideration parallels that of *Saprolegnia ferax* remarkably well. In the presence of galactose, *Phytophthora Cactorum* shows a marked decrease in rate of growth and on sucrose the growth is also lessened.

In figures 3, 4, and 5, the growth responses on Czapek's media are similar to those already indicated in figure 1. No marked deviation in growth rate, however, is shown on sucrose. On galactose in those three experiments the fungi *Physalospora Cydoniae* (FIG. 3), *Sclerotinia cinerea* (FIG. 4), and *Alternaria Solani* (FIG. 5), showed some decrease in the rate of mycelial growth, although this decrease in growth was not nearly so pronounced as was shown for *Saprolegnia ferax* (FIG. 1) and *Phytophthora Cactorum* (FIG. 2) when grown on the same kind of media.

Sclerotium Rolfsii (FIG. 6) grown on Czapek's medium showed a different or refractory vegetative growth response. On this medium with galactose sugar there was a marked increase in the rate of growth, measured linearly, and the quantity of mycelium produced was only slightly less in abundance when observed macroscopically in comparison to the quantity of growth produced on the contrasting carbohydrates. This, as the only exception encountered in this study, might bear further investigation.

Since in the second series of experiments peptone was added to an otherwise partially synthetic medium, the responses of the non-green plants to the carbohydrates present in this test would not be at all comparable to the responses shown in the first series of experiments. The series of experiments on Waksman's medium was made to discover if galactose exerted any inhibiting effects in the presence of peptone on such a medium. While there seemed to be less differentiation generally among the carbohydrates, however, in all cases tested it appeared that the rate of growth was depressed in the presence of galactose. Of the six composite graphs developed in this series of experiments to cover the six fungi selected, only one is given here in order to demonstrate the type of response found. This procedure is followed here because the experiments in this series were done as an explorative

venture and add nothing more to the interpretation other than what may be observed from a single type graph. Figure 7 introduced here, therefore, shows the type of response in the second series of

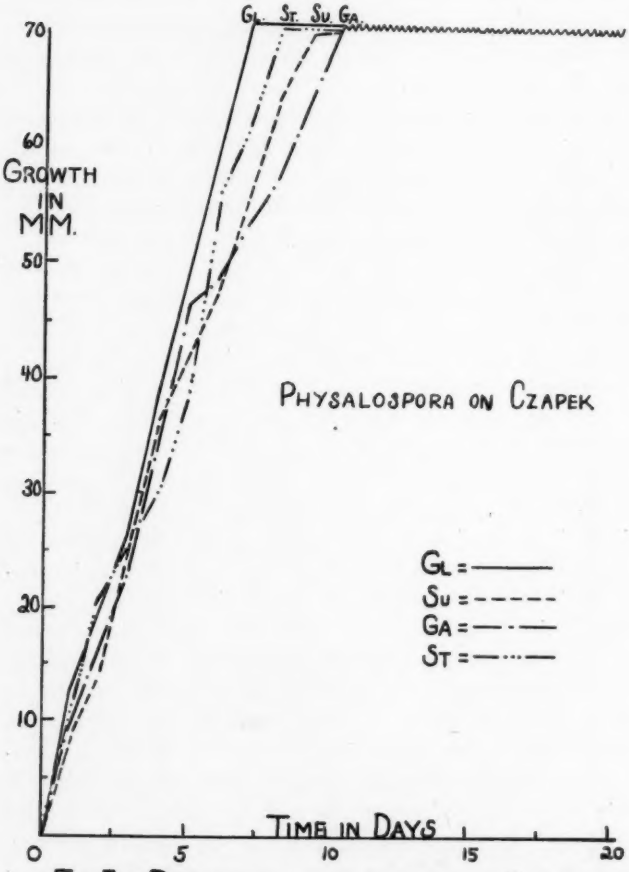

FIG 3 - PHYSALOSPORA CYDONIAE GROWN ON CZAPEK'S MEDIA

experiments as exhibited by *Phytophthora Cactorum* when grown on Waksman's medium with the specific carbohydrate ingredients; namely Wk-gl, Wk-ga, Wk-su, Wk-st.

The third series of experiments was performed with much the

same object in view as that followed in the second series. In the third series of experiments Sabouraud's medium were used in an exploratory approach. Sabouraud is a simple protein agar medium in which, through the complete suppression of any synthetic ingredients and with the introduction of peptone as a substitute, a suitable growth medium for fungi is obtained. Under these conditions in the fungi studied, galactose did not always appear to suppress the rate of growth. In some cases, however, decrease in rate of vegetative growth appeared sufficiently well defined to make record here. Figure 8 shows the graph of *Phytophthora Cactorum* on Sabouraud's media with the results against all four carbohydrates recorded, namely Sa-gl, Sa-ga, Sa-su, Sa-st. The graph shows, in general, lagging in the growth rate of *Phytophthora Cactorum* on these media though, as already indicated, this did not always hold true in the case of the other fungi examined.

As a matter of interest and clarification, two graphs are introduced here which give for a single fungus the complete results of all three media (namely Cz., Wk., Sa.) in relation to all four carbohydrates (namely gl., ga., su., st.) either in composite or simple combined form. Figure 9 shows the assembled results for *Saprolegnia ferax,* and figure 10 shows a similar compilation of data for *Sclerotium Rolfsii.* In figure 9 the graph lines representing the different carbohydrates for Sabouraud and Waksman media were so close together that they are combined in this plate to avoid congestion and confusion when interpreting the data on *Saprolegnia ferax.* In figure 10 which shows the graph lines for *Sclerotium Rolfsii,* all four carbohydrates for the three basic media remain distinct. The variance for galactose in *Sclerotium Rolfsii* on Czapek's medium is well illustrated.

The complete data for the width of hyphae is summarized in Table III. The measurements are given in microns. In the presence of galactose the width of the hyphae is consistently narrower. This change in width of hyphae is uniformly more pronounced on Czapek's medium, as would be expected, than on either Waksman's or Sabouraud's medium. Here again *Sclerotium Rolfsii* is somewhat refractory.

TABLE III.
WIDTH OF HYPHAE

		GLUCOSE	SUCROSE	GALACTOSE	STARCH
SAPROLEGNIA	1 Cz.	10	14	9	14
	2 Wk.	18	17	16	19
	3 Sab	20	19	15	22
PHYTOPHTHORA	1.	12	13	7	13
	2.	11	12	11	11
	3.	12	10	10	11
PHYSALOSPORA	1.	10	11	7	10
	2.	11	11	8	10
	3.	11	11	9	10
SCLEROTINIA	1.	11	10	9	9
	2.	11	10	9	10
	3.	16	18	13	12
ALTERNARIA	1.	11	9	8	9
	2.	10	10	9	10
	3.	13	12	11	10
SCLEROTIUM	1.	9	10	8	10
	2.	9	11	10	11
	3.	12	11	11	12

DISCUSSION AND CONCLUSION

Many references have been made in the literature calling attention to the toxic effect of galactose on the roots of green plants. The papers of Knudson (7) and (8) and that of Heinonen (4) probably make the most valuable contributions in this respect and offer the most searching study of the problem thus far in relation to green plants. In the main they both find parallel responses to galactose on the roots of green plants. Heinonen, however, de-

velops the question much further than Knudson but like Knudson offers no conclusive evidence in support of her findings.

Through their investigations they came separately to the conclusion that green plants were injured in a medium containing galactose. In the presence of galactose the roots of green plants showed discoloration, browning, abnormal structures and reduced growth. Knudson and Heinonen believed that the injuries occurring to the roots in the presence of galactose were due directly to the toxic nature of the sugar or indirectly to the oxidation products of galactose. In the absence of more recent literature to modify their explanation this point of view will be accepted.

In a similar manner, though with less frequency, articles from time to time have occurred in the literature relating to the effect of galactose on non-green plants. These references in the main have occurred indirectly during the investigation of a different problem. A thorough study of the literature on this aspect of the problem has been made by Horr (**5**) who discussed and summarized the different points of view. The reader is referred to this article for a summary of the situation relative to the effect of galactose on non-green plants. Because of the availability of this summary of the literature, only one or two references preceding the experimental work of Horr will be considered.

In an attempt to determine whether or not galactose is toxic to certain non-green plants as well as to certain green plants, Horr (**5**) grew the fungi *Aspergillus niger* and *Penicillium glaucum* for limited periods of time on synthetic media containing two per cent galactose. Under these conditions of growth he found both for *Aspergillus niger* and *Penicillium glaucum* that there resulted delayed germination of spores, reduced growth of mycelium, and finally the development of abnormal mycelial branches in the presence of galactose. Horr established these results on the basis of the rate of growth, estimated from the dry weight of the mycelium formed in liquid media.

In view of the experimental data obtained relative to slow spore germination, retardation of growth, and somewhat abnormal hyphal filaments when fungi are grown on galactose media, but without regarding these deleterious effects as evidences of toxicity when there is no actual indication of cell discoloration or cell

destruction, Horr (**5**) came to the conclusion that galatose is merely a poor source of carbon for fungi, and that the fungi *Aspergillus niger* and *Penicillium glaucum* merely utilizes galactose

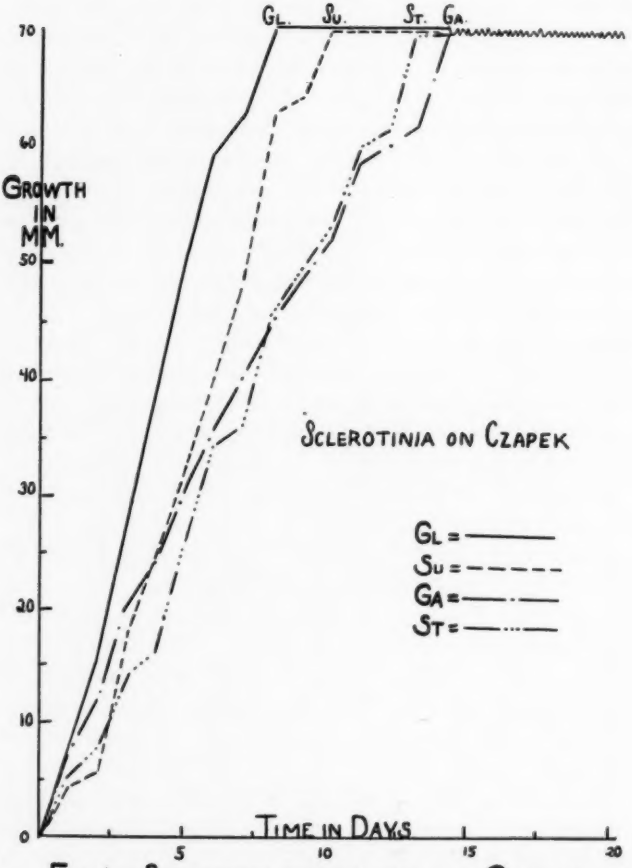

FIG. 4 – SCLEROTINIA CINEREA GROWN ON CZAPEK'S MEDIA

very slowly compared with the utilization of dextrose by the same fungi. Horr further found that galactose, when mixed with dextrose in suitable proportions, caused an acceleration in growth of the same non-green plants.

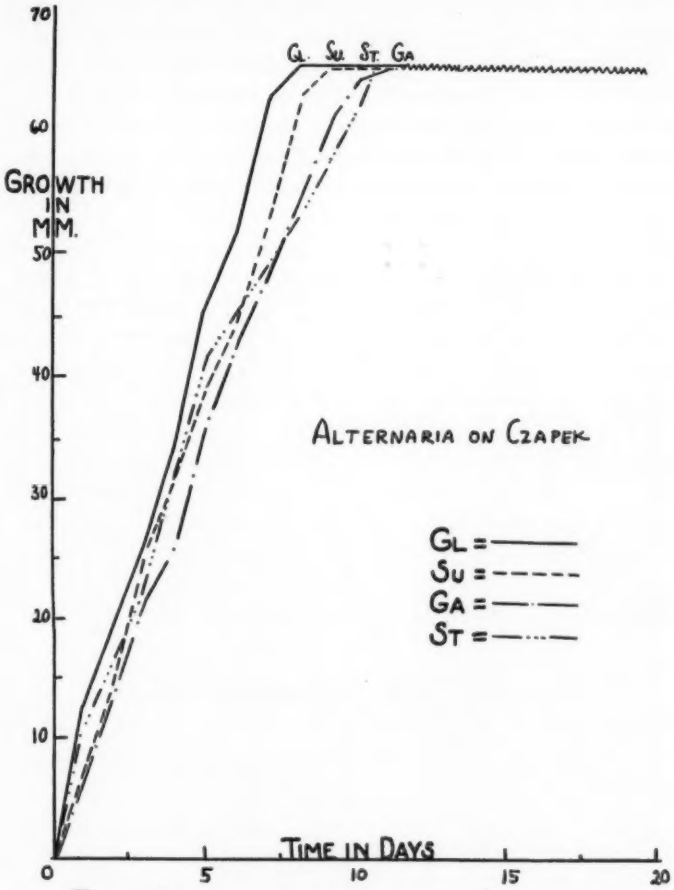

FIG. 5 - ALTERNARIA SOLANI GROWN ON CZAPEK'S MEDIA

The question of galactose toxicity on the roots of green plants, initiated first by Knudson (**7**), is expanded by Horr (**5**) who reviews the suggestions offered by Knudson in explanation of the toxic nature of galactose and, at least, shows in most instances the inapplicability of the explanation in relation to galactose toxicity toward non-green plants. However, Horr does not go far

enough in his generalizations, probably because of the limited nature of the illustrative material used in his experiments.

Although Horr worked entirely with a limited number and variety of fungi, the apparent different retarding responses observed may be typically characteristic of non-green plants, whereas toxic reactions may be the principal responses in green plants. These differences in plant responses to galactose evidently warrant

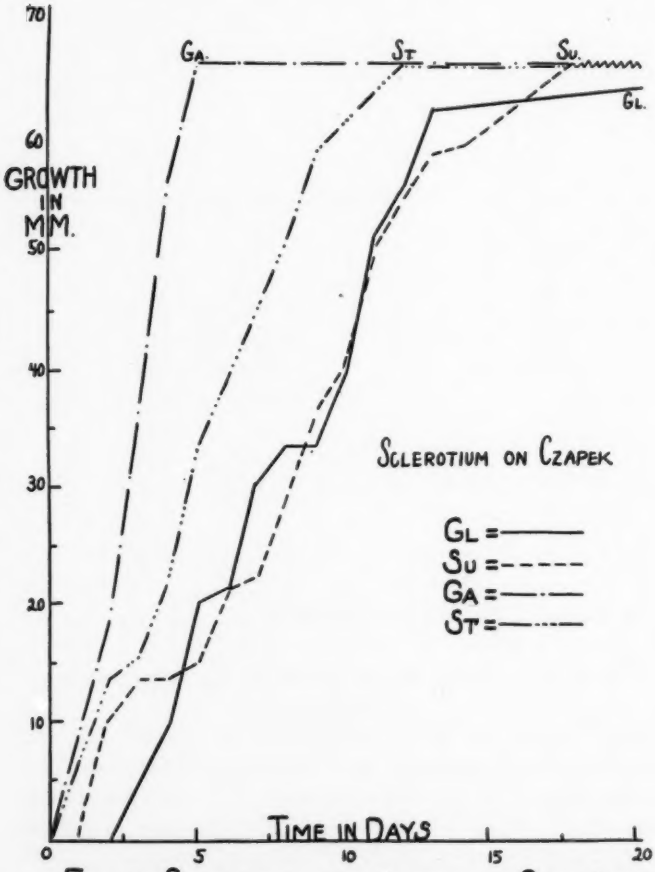

FIG. 6 – SCLEROTIUM ROLFSII GROWN ON CZAPEK'S MEDIA

the statement by Horr that non-green plants are more tolerant toward different sugars than are the green plants. The non-green plants indicate ability to utilize galactose as a source of carbon and do not exhibit particularly definite toxic effects as are specifically shown by the roots of many green plants under like conditions.

The work of Matsumato (**9**) in his findings from the growth of strains of *Rhizoctonia* on monosaccharide media supports the conclusions of Horr in that galactose was found to be available as a source of carbon to the fungous strains of *Rhizoctonia.*

Horr's conclusions are somewhat further supported by the findings of Coons (**1**) who, while working with the fungus *Plenodomus fuscomaculans,* found that the phases in the life cycle of the fungus were determined by the concentration of the sugar in the media due to the solubility of the carbohydrates present. Coons concluded that the differences in the growth form of fungi are connected with the amount of sugar supply available rather than with the specific nature of the sugar supplied.

In conjunction with other workers, therefore, the work of Horr appears to confirm the general belief that non-green plants respond less rapidly to and are affected less noticeably by the deleterious action of galactose than green plants, under similar conditions of growth.

The experimental results of the writer also show that galactose is a poor source of carbohydrates for non-green plants. The changes expressed by the fungi, moreover, in the presence of galactose, indicate that there is no ready availability of the galactose for the plant or that the sugar can only be absorbed with extreme slowness.

Grown on galactose media the rate of growth of the fungi used in these experiments, compared with the rate of growth when the fungi were grown on a non-galactose but carbohydrate medium, was always slower as is shown graphically in figures 1, 2, 3, 4, and 5 where the fungi were cultured on Czapek's medium, namely Cz-gl, Cz-ga, Cz-su, Cz-st. On the contrary, however, the fungus *Sclerotium Rolfsii* showed refractory responses to galactose, giving increased rate of growth, as is shown in the graph of figure 6, when cultured on Czapek's media, namely Cz-gl, Cz-ga, Cz-su, Cz-st. Is the variance shown by this fungus a peculiarity of the

mycelia sterilia group of fungi, or just an individual variation which might indicate the possibility that some non-green plants may show increased rate of growth in the presence of galactose,

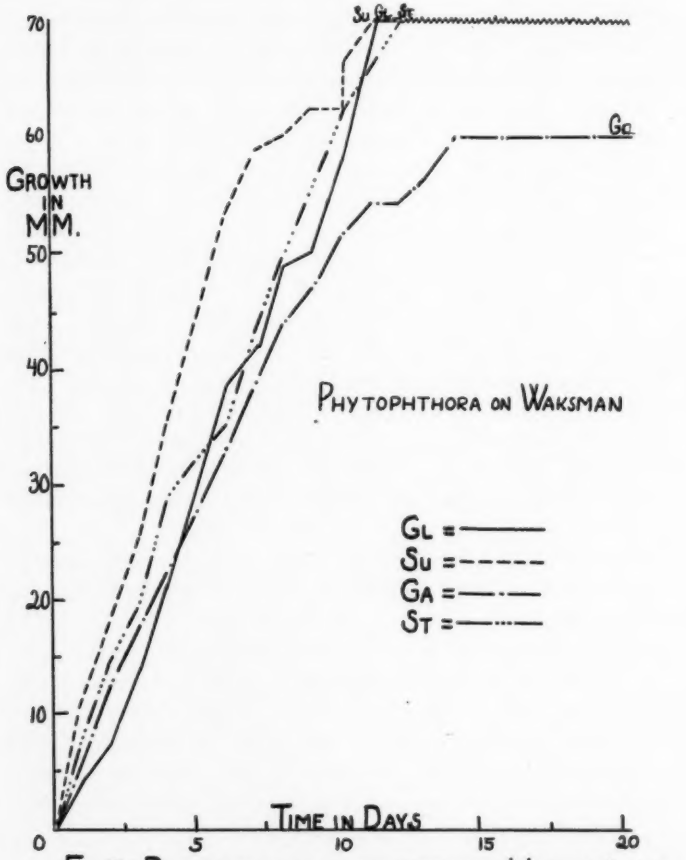

FIG. 7 - PHYTOPHTHORA CACTORUM GROWN ON WAKSMAN'S MEDIA

as has been reported in some instances for green plants in the presence of galactose. No explanation is offered for this unexpected behavior. The result in this case needs further investigation. The rate of growth in all cases was estimated by linear measurement on the basis of the diameter of the fungous-mat.

In most cases where estimates were made, the abundance of mycelium produced was always decidedly less when the fungi were grown on a galactose agar medium. This estimate was made macroscopically from a careful observation of the culture plants, depending solely on the quantity, thickness, and height of the mycelial mat.

On recording the data relating to the rate of growth during equivalent increments of time the microscopic field showed other additional features. First, there were no indications of discoloration, death of hyphal tips, nor disintegration of the fungous filaments on galactose media as was shown by the roots of green plants under similar conditions of growth, indicated by Knudson (**8**). In the second place, however, the mycelial filaments, shown in Table III, were regularly somewhat narrower on galactose media than the hyphal filaments when grown in dextrose agar media. Furthermore on galactose the mycelium showed many hyphal branches that were dwarfed and still other irregularities such as enlarged cells.

In the series of exploratory experiments made on Waksman's medium where, in addition to the sugars, the plant food peptone was added, the results in principle parallel those found when Czapek's medium were used. In consequence of the presence of peptone, the rate of growth of all six fungi on all four carbohydrates was increased. The lag in growth, however, of the fungi on media in which galactose as a sugar was used still appeared quite evident, but now on Waksman's medium the fungus *Sclerotium Rolfsii* showed no tendency to refractoriness. The behavior of the six fungi studied in this series is represented in this paper by one type—*Phytophthora Cactorum* (FIG. 7). This type fungus shows graphs for the four carbohydrates, one each for Wk-gl, Kk-ga, Wk-su, Wk-st.

The exploratory experiments made on Sabouraud's medium in which the sugars were added to media already suitable for the active growth of fungi, showed a situation in which the rate of growth of all six fungi on all four carbohydrates was increased, even more so than a similar increase in growth shown on Waksman's medium. The lag in growth in the presence of galactose was still very evident but again *Sclerotium Rolfsii* showed no refrac-

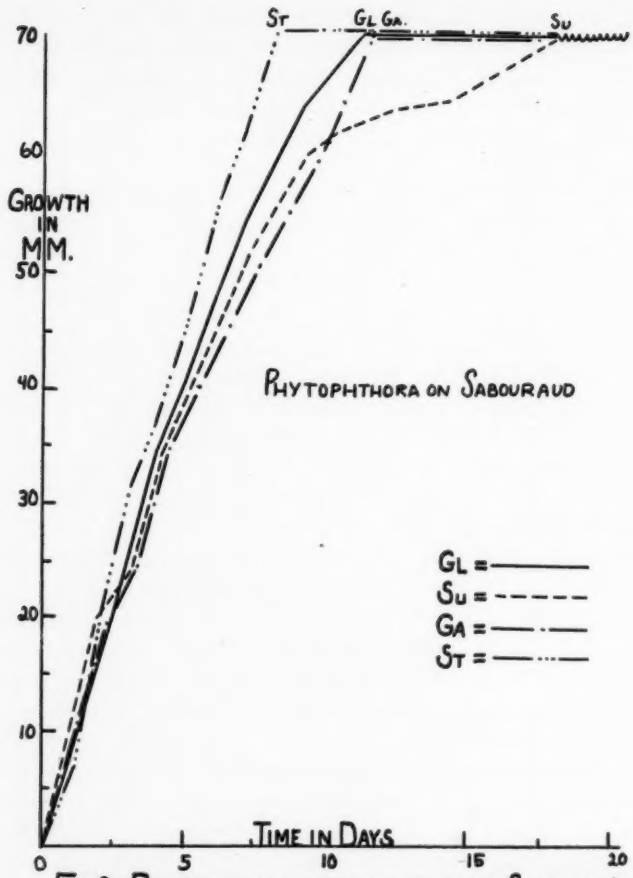

FIG. 8 - PHYTOPHTHORA CACTORUM GROWN ON SABOURAUD'S MEDIA

toriness. *Phytophthora Cactorum* (FIG. 8) was selected as a type to represent this series of experiments. In the graph the data from the four carbohydrates are assembled, namely Sa-gl, Sa-ga, Sa-su, Sa-st.

The graphs represented in figure 9 for *Saprolegnia ferax* show comparatively the rates of growth on the three basic media. The growth rates for the carbohydrates in the case of Waksman's and Sabouraud's media are here combined, while the results of rate of

growth on Czapek's medium for all four sugars are individualized by the graphs Cz-gl, Cz-ga, Cz-su, Cz-st. These graphs, shown in figure 9, indicate quite clearly the increase of rate of growth maintained on Waksman's and Sabouraud's media, as compared with the rate of growth made by the same fungus on Czapek's medium.

An attempt is made in figure 10 to show comparatively the 12 graphs of all four sugars on the three different media employed. Out of a possible six the one fungus, *Sclerotium Rolfsii,* is selected for this example. Here the rate of growth on all kinds of carbohydrate media used, is assembled individually on a comparative basis. On Czapek's medium with galactose as a component, *Sclerotium Rolfsii* is refractory. On Waksman's and Sabouraud's media the fungus is not refractory. The lag in growth rate of galactose in Waksman's medium is well marked by *Sclerotium Rolfsii* in figure 10. The rate of growth on Sabouraud's medium is very pronounced and growth with galactose as a sugar ingredient is also accelerated.

The width of the fungus hyphae in all six species shows a smaller diameter when measured in microns by the high objective of a compound microscope in those experiments in which the fungus grew on a galactose ingredient medium. This was true whether the fungus was cultivated on Czapek's, Waksman's, or Sabouraud's media. The distinction is illustrated in the results of this experiment and is shown by the barred numbers in Table III.

That other fungi exhibit retarded growth rate in the presence of galactose compared with the rate of growth in the presence of glucose, was recently very clearly demonstrated by Kinsel (**6**) for the corn inhabiting species of *Diplodia.* In a period of three weeks, according to Kinsel, the quantity of mycelium produced by *Diplodia Zeae* on Richard's synthetic medium with galactose used as the source of carbohydrate, was only half the quantity produced when glucose was added as the source of carbohydrate.

In view of the data compiled by Knudson and Horr and the inferences or conclusions they draw from these data, together with the results of the experiments reported in this paper, the writer is inclined to believe: First, that non-green plants are less suscep-

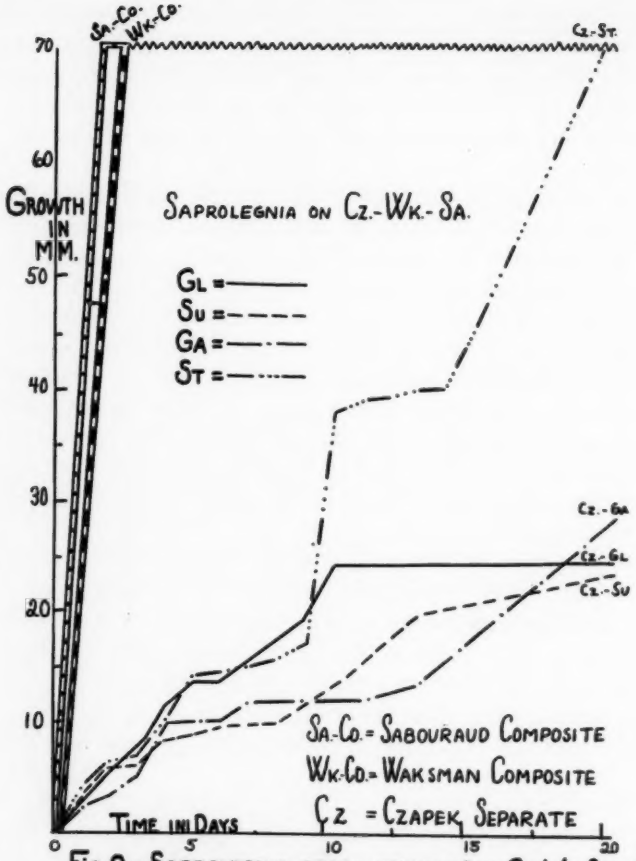

FIG. 9 - SAPROLEGNIA FERAX GROWN ON CZ-WK-SA MEDIA

tible than green plants to the inhibiting effect of galactose in their immediate environment; and second, that galactose is not toxic to non-green plants as it appears to be, from well established experimental data, towards the roots of green plants; furthermore, that galactose as a source of carbohydrate for non-green plants is somewhat less available than glucose under the same growth conditions, and that galactose is less readily absorbed than glucose by non-green plants. Galactose is utilized by plants with greater diffi-

culty than glucose according to Maximov (10) because of its somewhat different atomic configuration.

The fact that the effect of galactose on non-green plants is different from its effects on green plants is not to present a divergent

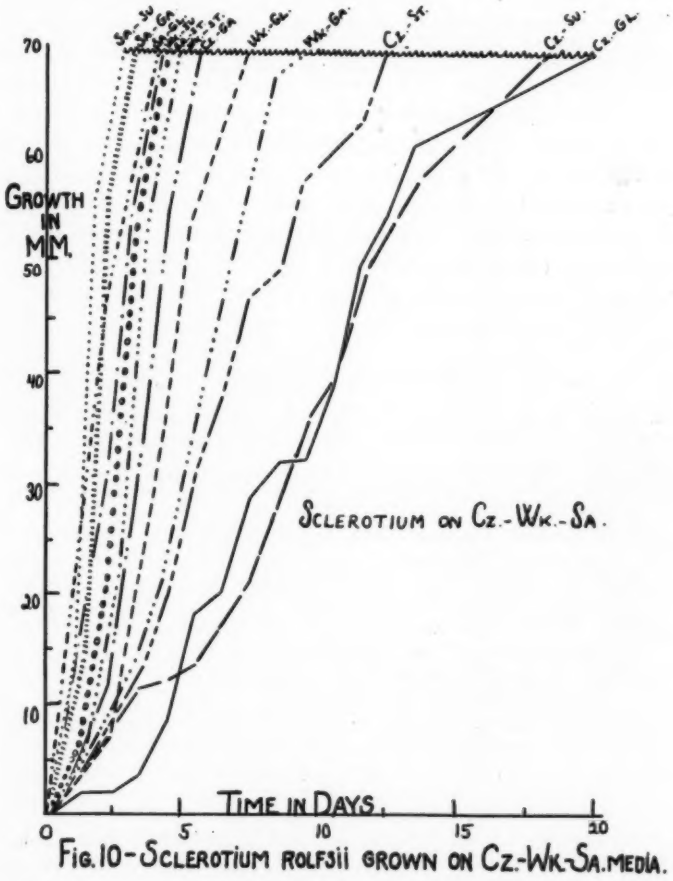

FIG. 10—SCLEROTIUM ROLFSII GROWN ON CZ.-WK.-SA. MEDIA.

point of view. Green plants are adapted to the reception of foods as carbohydrates of a limited variety, principally glucose since they depend for sugars normally on a common photosynthetic process, thereby obtaining sugars uniformly in a specific form. For that

reason the green plant may be less tolerant toward sugars of a diverse nature. Non-green plants, on the contrary, appear to be more tolerant toward many different sugars than their green plant relatives. In this instance the non-green plant is dependent on the utilization of the digested products of highly organized organic substances from a great variety of outside sources, obtained solely through its absorptive structures. Therefore, the non-green plants may have become adapted to the utilization of different available simple sugars, resulting from the digestion of complex organic compounds. This view is supported by the investigations of Euler (2) on yeast, where it was found that a yeast was able to adapt itself to galactose as a source of carbon and in so doing increased its ability to ferment galactose much faster than it could ferment other sugars.

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SPECIES OF CLADOSPORIUM ON TOMATO AND THE ALLERGIC RESPONSE IN MAN AS AN AID TO THEIR IDENTIFICATION [1]

EMIL F. GUBA [2] AND FRANCIS M. RACKEMANN [3]

(WITH 1 FIGURE)

Cladosporium fulvum Cooke was described in 1883 by M. C. Cooke (**5**) for the fungus causing disease of tomato leaves sent to him from North Carolina. Since then, the organism has been recognized all over the world as a serious leaf parasite of the tomato, especially tomatoes grown in greenhouses. Also, the fungus has been reported to cause rot in the stem end of the fruit by invasion from infected blasted blossom parts (Gardner (**8**), Makemson (**13**)).

In 1887, Plowright (**19**), described a leaf mold disease on tomato with spores of a beautiful violet tint which, for that character alone, he named *Cladosporium fulvum* Cooke var. *violaceum*. Voglino (**27**) in 1912, unaware of this previously described violet variety, reported a similar aberrant form from Italy to which he gave the same name. In Italy, this violet variety was further encountered by Savelli (**22**) who made of it the basis of a paper to show that the violet color was a constant character and not incidental, and that it was to be regarded as a variation or mutation. Hasper (**11**), however, declared the variety to be unjustified in view of the fact that the violet color could be produced by a modification of environmental factors, especially the alkalinity of the substratum. Makemson (**13**) reported that in culture the fungus

[1] The writers are gratefully indebted to Dr. W. W. Diehl, U. S. D. A., and Dr. G. D. Darker, Harvard University, for assistance in bringing to their attention some of the pertinent literature referred to in this paper.

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manifests a beautiful purple color and that on infected leaves this coloring matter is located in the conidia and conidiophores. Diffused light and dryness were factors contributing to its development. Schaffnit and Volk (**23**) noted changes in the color of the fungus on tomato foliage by varying the nutrition of the host plant. One of the present writers has observed the rich purple or violet color from time to time in older cultures and on infected tomato foliage but has never regarded it as of taxonomic significance because of its variability under changing conditions. In culture the fungus is rather slow growing and on cornmeal agar (Difco) the young single-spore colonies show a distinct yellow-brown or tawny color. Later, violet-purple and even crimson colors appear. The varietal name *violaceum* suggesting a violet form, therefore, can have no standing in the literature.

Other names for the tomato leaf mold fungus have appeared in the literature to cause confusion. In 1899 McAlpine (**14**) from South Australia reported *Cladosporium Solani* McAlpine as new and destructive to tomato leaves. Judging from the description of the disease, the fungus could have been none other than tomato leaf mold caused by *Cladosporium fulvum* Cooke, which is now generally recognized in the literature from Australia. The name was used more recently (1919) in reporting a serious occurrence of tomato leaf mold in greenhouses near Indianapolis, Indiana (U. S. Dep. Agr. Pl. Dis. Bull. 3: No. 4, 57, Aug. 1, 1919). More recently, Esmarch (**7**) and two years later, Ludwig (**12**) used the name *Cladosporium fuscum* Link in their accounts of the tomato leaf mold disease (Braunfleckenkrankheit) in greenhouse culture in Germany. The species *fuscum* was described by Link (Linné. Sp. Pl. p. 40) in 1824 on stems of *Rosa* in Germany and more recently reported in the United States on leaves of wild and cultivated rose (Anderson et al, **2**). There is no literature to justify the substitution of *C. fuscum* Link for *C. fulvum* Cooke, and the use of the incorrect name seems to be authors' errors rather than attempts to justify the validity of the name.

Green tomatoes arriving on the Boston Produce Market from California often show considerable rot associated with what has been reported to be *Cladosporium fulvum* Cooke. Colored plates typical of this rot are shown by Ramsey and Link (**20**), and ac-

counts of the rot and of the environmental relations of the organism are given by these authors and by Nightingale and Ramsey (**15**). These are, however, totally unlike our conception of *Cladosporium fulvum* Cooke and of the rot which it produces in tomatoes. A review of the literature shows further confusion relative to the problem.

Plowright (**18**) described *Cladosporium Lycopersici* associated with what we know as blossom end rot of tomato. The same disease and organism were reported by Smith (**25**). Reinmuth (**21**) illustrated and described a similar rot at the blossom end and on incubation of the tomatoes obtained a dense growth of fungus which he identified as *Cladosporium fulvum* Cooke. Perotti and Cristofoletti (**17**) noted *Cladosporium herbarum* Link associated with dark-olive spots on green and ripe plum and pear tomatoes. Successful artificial infection of fruit removed from the plants was obtained by inoculations with the fungus from pure culture. Some decay was manifested after a month from the time of inoculation and only inoculations through injuries in the pericarp were successful. Tomatoes of large-fruiting varieties were resistant and none were ever found infected in the market stalls. Likewise, fruits of susceptible sorts were resistant so long as they were growing on the plants. Halsted (**10**) reported a destructive rot of green and ripe tomatoes which he attributed to *Cladosporium fulvum*. His description of the rot which followed inoculations with spores of *Cladosporium* from tomato leaves clearly suggested some other causal organism. Little significance may be attached to this report since Halsted also entertained doubt as to the exact cause of the rot. Plowright (**19**) described spots which gave the tomato fruits a mottled appearance on reddening. No fungus was found with these spots but *Cladosporium* was suspected because of the destruction of the foliage by *Cladosporium fulvum*. Since the stems were streaked, it is possible as Gardner (**8**) has suggested that the trouble could have been a virus disease.

The various accounts of *Cladosporium* associated with tomato fruit rots, blossom end rot and sunscald suggest the fungus *Cladosporium herbarum* (Pers.) Link, usually regarded as the type species, certainly not *C. fulvum* Cooke. *C. Lycopersici* Plow.

may be regarded as a synonym of *C. herbarum*. A culture of *Cladosporium herbarum* Link from wheat supplied by the Centraalbureau voor Schimmelcultures (Baarn, Holland) is similar to the *Cladosporium* from green rotted tomatoes from California gathered on the Boston market, and to cultures from rotted peppers and tomatoes supplied by Dr. G. B. Ramsey, United States Department of Agriculture, and to a culture of *Homodendron* sp. supplied by Dr. S. M. Feinberg of Northwestern University Medical School.

The failure of the fungus isolated from green rotted tomatoes to produce decay in our inoculations of green tomato fruits and the fact that Nightingale and Ramsey (**15**) obtained very little decay of tomato fruits with it in their work, lead us to conclude that this species is a saprophyte or at the most a very weak pathogen on tomato fruits. It seems clear that the brown and violet colored species on tomato leaves (*fulvum*) is totally distinct from the dark-olive colored species (*herbarum*) on decaying green tomatoes in transit or following sunscald and blossom end rot.[4]

The chief object of this paper is to present another method of differentiating closely related species and varieties of fungi. It is now recognized that fungi of different kinds, especially the imperfect fungi, may cause asthma in certain persons who have developed a hypersensitiveness to their spores. This possibility was first suggested in 1924 by Cadham (**3**), who reported three cases of asthma in farmers working with wheat contaminated with rust caused by the fungus *Puccinia graminis* Pers. More pertinent, however, is the report by Cobe (**4**) in 1932 who recognized that the violent asthma in his patient was due to the spores of *Cladosporium fulvum* to which the man was exposed by his work as a greenhouse tomato grower. When an extract of the mold growth was made and applied to a scratch in the patient's skin, a raised wheal with surrounding erythema appeared within a few

[4] It seems desirable here to call attention to a few other errors of nomenclature which have appeared in the literature. Ellis (**6**) reported *Cladosporium lycoperdinum* Cooke on tomato fruits. This species was originally described on *Lycoperdon*, not *Lycopersicum*. Stevens (**26**) noted *Cladosporium scabies* Cooke on tomato but this is a textbook error. These errors have been carried along in the literature; Seymour (**24**), Norton (**16**). In both instances the better name would appear to be *C. herbarum* (Pers.) Link.

minutes, and subsequently a course of treatment with the extract resulted in a reduction of the degree of sensitiveness such that asthma no longer occurred on exposure to the parasite.

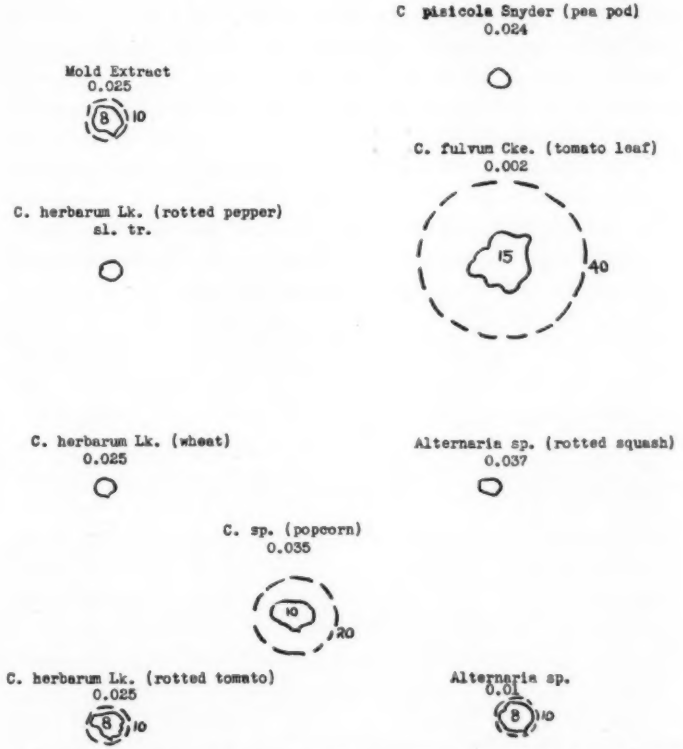

FIG. 1. Intracutaneous tests on E.F.G. sensitive to *Cladosporium fulvum* Cooke. Readings in 20 minutes. Figures under fungous names indicate milligrams total nitrogen per cc. Figures on diagrams indicate average diameter of urticarial wheal (solid line) and of surrounding erythema (dotted line).

This condition of sensitiveness to *Cladosporium fulvum* is not common, but nevertheless, several cases similar in every way to that described by Cobe have come to our attention. Feinberg and Debtwater also have seen similar cases.[5] In April 1936, Guba

[5] Indicated in personal communication to one of the authors.

(9) published a short note with a description of the symptoms as follows: "The patient is overcome with spasms of violent sneezing, irritation of the skin, eyes, and mucous membranes, wheezing and discomfort at night, difficulty in breathing, irritation across the chest. These symptoms are associated with much flow of mucous from nose and throat which may continue like a cold for several days. Recovery is slow and gradual. On further exposure to plants infected with the fungus or in packing tomatoes gathered from infected plants, all symptoms return again."

Would patients of this sort show skin tests to other species of *Cladosporium* as well as to *Cladosporium fulvum?* The question was of considerable academic and perhaps practical importance, and to answer it, pure cultures of species of *Cladosporium* and other organisms were obtained as follows:

Cladosporium pisicola Snyder from inside of pea pod from Cornish, Maine. Identity of fungus confirmed by Snyder (Phytopath. **24**: 890 and Errata; Plant Dis. Surv. **20**: 301). Submitted by Dr. Donald Folsom.

Cladosporium herbarum Link. Bennet Str. V from wheat. Culture obtained from Centr. Bur. v. Schimmelcult., Baarn, Holland.

Cladosporium fulvum Cooke from tomato leaves in greenhouse, Waltham, Mass., by E. F. Guba.

Cladosporium sp. from popcorn kernels, Maine. Submitted by Dr. Donald Folsom.

[6] *Cladosporium herbarum* Link from rotted California pepper, Chicago Terminal Market. Submitted by G. B. Ramsey as no. *2485.*

[6] *Cladosporium herbarum* Link from rotted California green tomato on Boston Produce Market by E. F. Guba.

Mold extract (origin unknown)

Alternaria sp. (origin unknown)

Alternaria sp. from decayed squash. Concord, Mass., Dec. 20, 1936, by E. F. Guba.

Each culture was grown on potato agar in a 250 cc. Erlenmeyer flask. After about two weeks, the growth was treated by adding

[6] This organism on pepper and tomato has been mistaken in the literature for *Cladosporium fulvum* Cooke.

25 cc. of isotonic buffered phosphate solution called "Coca's fluid" to the flask and letting it stand for several hours, scraping the growth with a spatula from time to time. Then the extract was filtered through paper and finally sterilized by passage through a Seitz wafer. The total nitrogen was determined and each extract was so adjusted with the salt solution that it contained about 0.02 mg. N per cc. Skin tests were then made with each extract, by the intracutaneous method, injecting tiny amounts with a needle between the layers of the skin. The reactions which developed in fifteen minutes are indicated diagrammatically in figure 1.

The differences in the cutaneous responses are well marked. *Cladosporium fulvum* gave a large irregular wheal, and this particular test was made with a higher dilution, containing only 0.002 mg. N. Three of the *Cladosporia* gave entirely negative tests and so provide a valuable control. Particular interest attaches to the tests with the two strains of *Cladosporium herbarum* Link. The strain obtained from the rotted California green tomato does give a small response while the other strain from rotted California pepper is negative. However, the differences are slight and may well be due to the technique of the tests including some variation in the nitrogen content of the particular extracts used. Control tests with other molds extracted in the same way are essentially negative.

These clinical observations have been made so far on only one case but the results are so striking that they "must be" reported! Whereas the size of reactions in other cases may vary considerably, there is no doubt about the fact that skin tests in susceptible individuals show clear-cut differences between the extracts of different species of *Cladosporium*. This biologic test appears to be a new method by which varieties of fungi can be distinguished one from another and with considerable certainty.

Another biologic method was used by Almon and Stovall (1) in their study of *Monilia* and other yeast-like organisms. They immunized rabbits with a series of intravenous doses of an extract obtained by washing malt agar plates with a solution containing 0.50 per cent NaCl and 0.10 per cent Formalin. The serum of the treated animals was used for various agglutination and absorp-

tion procedures. A good deal of crossed reaction was found and the reactions were not always specific for the particular strain employed. It is recognized, however, that no test for circulating antibodies can reach the delicacy of the test for fixed antibodies in the skin. Obviously a direct comparison of the two methods would make an interesting experimental study.

SUMMARY

Cladosporium fulvum Cooke var. *violaceum* Plowr. and *C. Solani* McAlpine are synonyms of *C. fulvum* Cooke. The fungus is the cause of the leaf mold disease of tomato. On rare occasions it causes a rot only in the stem end of the fruit following infection of the blossom parts. The color of the fungus in culture and on its host is variable under changing conditions and therefore is not a distinguishing character.

Cladosporium fuscum Link, originally reported on rose stems, is different from *C. fulvum* Cooke. The name can not be used for the tomato leaf mold fungus.

C. Lycopersici Plowr., *C. fulvum* Cooke and *C. herbarum* Link have frequently been identified with the decay of tomato fruits in transit and market stalls and following sunscald and blossom-end rot. *C. fulvum* Cooke does not occur in this manner and accounts of decay of tomatoes identified with this fungus clearly show that the wrong name has been used. *C. Lycopersici* Plowr. appears to be the same as *C. herbarum* Link, generally regarded as the type.

Cladosporium scabies Cooke was originally a textbook error. *C. lycoperdinum* Cooke once reported on tomato fruits was originally described on *Lycoperdon* sporophore and has nothing to do with the genus *Lycopersicum*. *C. herbarum* would seem to be the better name in this case.

C. fulvum Cooke is the cause of a violent asthma in human beings and skin tests with extracts of the fungus produce a marked reaction.

Intracutaneous skin tests with extracts of several different species of *Cladosporium* on a susceptible individual show well-marked differences, and a very great difference between *C. fulvum* Cooke and *C. herbarum* Cooke, the former from greenhouse tomato

leaves and the latter from rotted California tomatoes and peppers.

The results point to the fact that individuals allergic to fungi show strong differences to extracts of species in the same genus and that this biologic test appears to offer another method by which closely related fungi may be distinguished one from another and with considerable certainty.

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MORPHOLOGY AND CYTOLOGY OF GUEPINIA SPATHULARIA

SISTER MARY CECILIA BODMAN

(WITH 44 FIGURES)

Two-spored basidia occur in a number of Basidiomycetes, among which may be mentioned the common cultivated form of *Agaricus campestris, Amanita bisporigera, Craterellus cornucopioides* and a number of Gasteromycetes. In all of these cases, however, the two-spored condition is exceptional, the great majority of the species of the Agaricales having four-spored basidia; the Gasteromycetes, while more variable in this respect, frequently have more than four. The Dacryomycetaceae is the only extensive group in which the basidium is consistently two-spored. It follows that members of this family afford particularly favorable material for studies which attempt to correlate the two-spored with the more usual four-spored condition. The questions to be considered are: Does nuclear fusion take place in the young basidium, and is it followed by meiosis in the usual manner? If so, what disposition is made of the resulting four nuclei? Do two nuclei pass into each spore? Does the basidium produce a second crop of spores? Or do two of the nuclei remain within the basidium, degenerating with it? Previous reports are far from agreeing in their answers to these questions and it seems desirable to attempt to throw more light upon the problem.

The species chosen, *Guepinia Spathularia,* was selected because it is exceedingly common in North America, it is a typical member of the Dacryomycetaceae and it has not previously been studied cytologically. Although apparently not indigenous to Europe, its taxonomic position has been considered so frequently that it may be said to be universally known. In addition, an abundant supply of early stages was at hand, permitting observations upon the morphological development of the basidiocarp, to which little attention has been paid in tremellaceous fungi. The reasons for

adopting the name here applied have recently been discussed at length (**16**).

A complete review of the literature treating this species has not been attempted. Originally described by Schweinitz as a *Merulius,* it was given the name here adopted in 1828 by Fries (**8**); in the United States, Burt (**3**) and Coker (**4**) have published descriptive accounts. Fisher (**7**) and Martin and Huber (**17**) have described its occurrence in Iowa. Fisher's paper (**7**) also contains a good account of the morphology of the mature sporophore. Creager (**5**), who germinated the spores and grew the mycelium upon culture media, gives the method of germination of the spores, the development of the mycelium, and a brief account of an experiment upon the pigmentation.

Buller's account (**2**) of the discharge of the spores in the Basidiomycetes, and of the subsequent collapse of the basidia is especially valuable because his experiments were performed upon living material.

Levine (**14**) published in 1913 a tabulated account of the cytological studies made upon the Basidiomycetes up to that time. That portion relating to the Dacryomycetaceae seems to be quite complete. Those papers are reviewed briefly here, and the two papers published since Levine wrote in 1913 added to the list.

The first cytological studies upon the Dacryomycetaceae were made by Dangeard in 1895 (**6**). He studied *Dacryomyces deliquescens* Bull. and *Calocera viscosa* Pers., noting the fusion of the primary nuclei and the organization of the nucleus. However, he observed only one division of the fusion nucleus, and stated that one nucleus passed into each of the two spores.

In the same year Istvanffi (**11**) described *Dacryomyces chrysocomus* (Bull.) Tul. noting two divisions of the fusion nucleus, but, interestingly enough, failed to mention the fusion of the primary nuclei. He followed the passage of the nuclei into the two epibasidia, and noted that only one nucleus passed into each spore. He then postulated the formation of a second crop of spores.

Juel (**12**), studying *Dacryomyces deliquescens* (Bull.) Duby, described the details of the meiotic process. His account of nuclear division and spore formation agrees with that of Istvanffi, except that he does not mention a second crop of spores.

Maire's account (**15**) of the cytology of *Dacryomyces deliquescens* also confirms Istvanffi's findings. He gives the chromosome number as two. He says that in *Calocera cornea* Fries either two or four nuclei may be formed. If two, only one set of spores is borne; if four, then two sets are produced upon each basidium.

Wager's account (**20**) of an unspecified *Dacryomyces* agrees with those of the earlier workers. He dismisses the possibility of a second crop of spores as based upon insufficient evidence, and states that it is not impossible that two nuclei pass into each spore. He gives four as the chromosome number.

The most recent worker upon this family, E. M. Gilbert (**10**), used three different *Dacryomyces,* but did not give the species. He found centrosomes to be present, gave four as the chromosome number, and stated that two nuclei remain in the hypobasidium and degenerate.

The work of these students shows that there are present in the young dacrymycetaceous basidium, as in general among the basidiomycetes, two primary nuclei, which fuse and immediately divide meiotically, forming four nuclei. Two spores are produced, apparently uninucleate. These spores may be one, three or more septate. The final disposition of the four nuclei is not so definitely established. The assumption of Istvanffi and Maire that two crops of spores are produced seems to be an inference not based upon actual observation, inasmuch as the basidium collapses after spore production, and becomes practically invisibile. Wager introduces another possibility—that two nuclei pass into each spore. This alternative is also suggested by Rosenvinge (**18**) for *Craterellus cornucopioides.* E. M. Gilbert stated that two nuclei remain behind and degenerate with the basidium. Juel's paper implies the same, although he does not make a definite statement to that effect. Gilbert appears to be the only one who has actually seen the degenerating nuclei. It is unfortunate that he does not give the species of *Dacryomyces* he studied.

In order to determine the most satisfactory method of killing and fixing the material, sporophores were fixed in each of the following solutions: formalin-acetic acid-alcohol, weaker Flemming's, Bouin's solution, and Allen's modification of Bouin's solution.

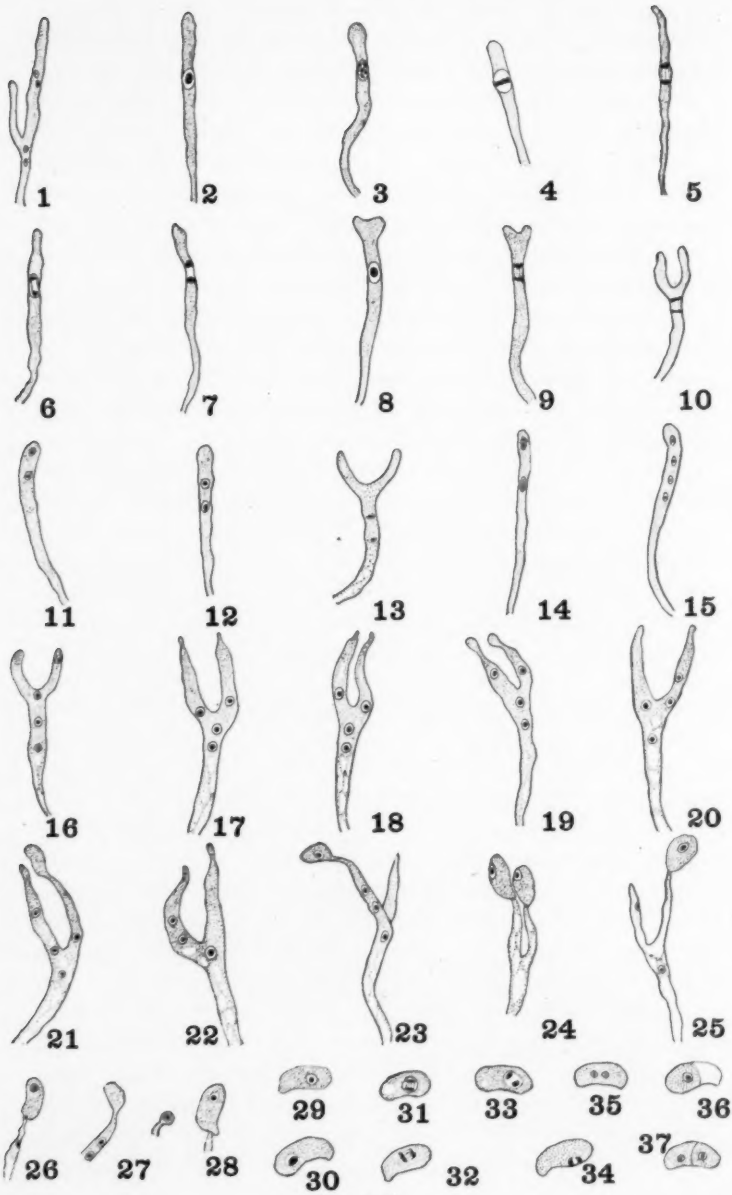
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FIG. 1–37.

The material was left in the fixing solution for from 24 to 48 hours, and washed in running water. It was then dehydrated in a butyl alcohol series. The materials are ordinarily left in the lower concentrations for one hour, but this was found to be inadequate. A weak solution of Fast Green that had been placed in the 60 per cent alcohol penetrated scarcely more than the cortex of the fructification. Subsequent material was accordingly left in each concentration for a half day or longer, and for 24 hours in 100 per cent butyl alcohol.

The material was embedded in paraffin with a melting point of 52° and cut at 5 μ. None of the material which had been fixed in Bouin's solution was usable. The sections were so badly broken that they could not be mounted on the slides, and were therefore discarded. As identical methods of embedding and dehydrating had been used, the failure was attributed to the fixing solution.

The sections were stained in Flemming's triple, or in Haidenhain's haematoxylin. The haematoxylin was the more satisfactory for showing nuclear detail, and was employed for all the slides used in the cytological study. Sections were left in 5 per cent iron alum for from two to five hours; washed thoroughly in distilled water, and transferred to the haematoxylin. Sections left for two hours in the mordant were stained for five hours, while those that had remained in the mordant for five hours were stained for a shorter period, usually for three hours or less. The latter proved to be the more satisfactory.

After the slides were removed from the staining dishes, they were washed in tap water and then in several changes of distilled water. Destaining was done with 2 per cent iron alum. No counterstain was used. While the basidia were in all cases unequally stained, in each section a certain number were satisfactory for the study of nuclei. In the best preparations the cytoplasm appears as a pale gray background, with the nucleus showing as a dense, black body. Material which had been fixed in Allen's modification of Bouin's solution furnished the sections which have proved most useful. Sections fixed in weaker Flemming's solution or in FAA were also satisfactory.

The material used in the morphological study was cut at 10 μ

and stained with Fast Green SF or with Phloxine. An aqueous solution of acid fuchsin was also employed, but was not successful. The hyphal walls seemed to be impervious to most dyes, and often only one or two sections on a slide absorbed the stain. A few slides were stained with Delafield's haematoxylin to show the nuclei and the septa, as this relationship is not shown by Haidenhain's stain.

Guepinia Spathularia is entirely saprobic and appears typically upon decorticated wood, growing in lines from cracks or upon the surface. The specimens used in this study were collected in Iowa City, upon decaying apple wood. The fructifications are firm and somewhat tough in consistency, bright-orange in color, and are divided into a distinct stalk and head. They vary from less than five to about fifteen millimeters in height, and have the spathulate form which gives the specific name. Some sporophores were clavate in shape, while others were almost lobed.

The stipe is somewhat darker than the head, and slightly tomentose. The orange or yellow head is gelatinous in appearance, in spite of its tough consistency, and becomes very thin at the edges. As the fruit body becomes larger it droops over toward the wood upon which it is growing. The surface which is toward the wood becomes marked with one or more cantharelloid folds, and upon this inferior portion the hymenium is borne.

The hymenium matures very early. Specimens were found in which little or no differentiation of stipe from head was evident, but which nevertheless were shedding spores. The fructification is xeric, becoming dormant during dry periods, and reviving and again shedding spores in moist weather, so that spores are produced over a long period of time. Morphological differentiation may no doubt continue, even though the reproductive portion is mature.

The white mycelium is not visible upon the surface, but may be found just beneath it. A study of prepared sections shows that this mycelium may grow transversely through the medullary rays, or longitudinally through the vessels (FIG. 38). Within the vessels the hyphal strands are so closely crowded together that little of the relationship of one hypha to another may be seen, but in the rays they tend to be independent and parallel, so that the crossing

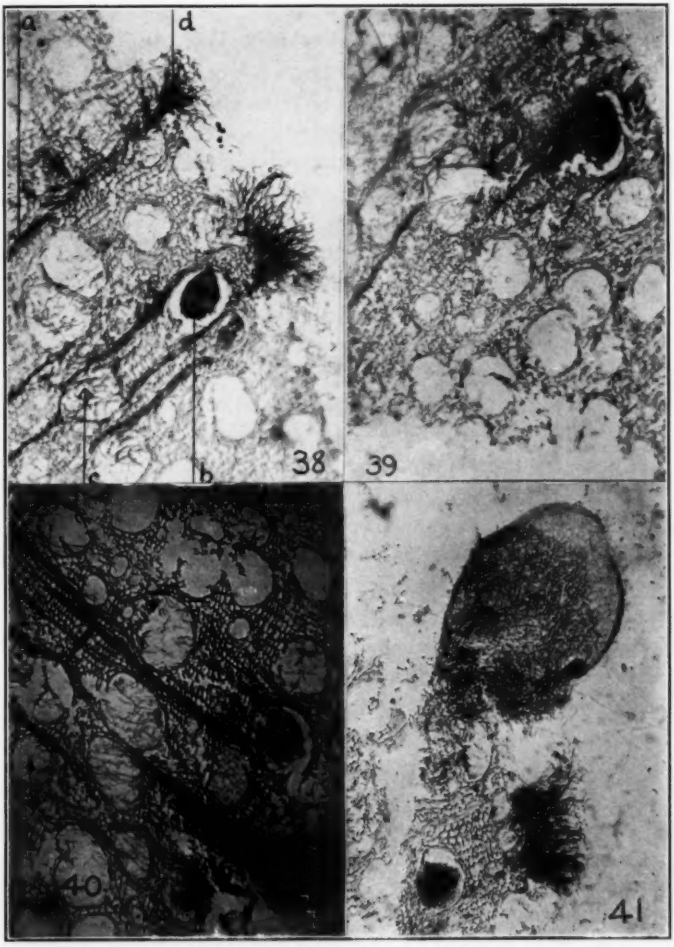

FIG. 38–41.

and intertwining of the threads, and the numerous anastomoses may be seen. The hyphae measure about 1.5–2.5 μ and are smooth, with binucleate, somewhat elongated cells, which are swollen at the partitions. No clamp connections have been observed.

The fructification may be formed directly from the strands of mycelium which grow through the medullary rays. The loose ends of the hyphal strands separate when they reach the surface, branching and spreading in a fan-like manner (FIG. 38, 41, 42). The hyphae in the interior of the mass become more and more closely united, branching and anastomosing freely (FIG. 40, 42). The sporophores now consist of massed clusters of hyphae, surrounded by loose mycelial threads. As they lie upon the surface of the wood, they appear to be tiny, spherical, bright-orange masses, embedded in a dense white tomentum. This tomentose covering eventually disappears.

If the hyphae which are growing through the medullary rays encounter a vessel whose walls have been weakened by decay, they will mass within this tube, forming a structure which in appearance resembles the beginnings of a sporophore (FIG. 39). However, only the most immature stages were noted, except when the vessel in question was close to the surface. The hyphae then grew out into the air, and there completed the formation of the fructification (FIG. 43).

There seem to be two factors attendant upon the formation of the fruit bodies. One is the release of pressure, the other is the presence of air. The presence of light may also be a factor in development—at least, sporophores developed in the laboratory during the months of November and December were distinctly etiolated; the etiolation being evidenced by extreme elongation of the fructification, which assumed a cylindrical shape, and failed to develop a hymenium. There was no conspicuous change of color.

The cortex is differentiated very early. The first step is a reduction in size in the hyphae which form the outer portion of the sporophore. These smaller mycelial threads form a layer which is regular in depth and which surrounds the exposed portion of the sporophore. The reduction in size is abrupt, so that the division between the larger medullary and the small cortical hyphae is very distinct. The gelatinous matrix in which the hymenium, when it appears, will be embedded, also is present. There is at the same time, little or no evidence of a stipe, and almost no alteration in the appearance of the medulla (FIG. 41). Since the

cortical hyphae are the only ones which have been changed, it may be that the tremellaceous sheath is formed from them. The sporophore at this time presents the translucent, distinctly gelatinous appearance of a tremellaceous fungus.

In the next stage to be examined, the hymenium was already differentiated and spores had matured, although the sporophore was still ovate in shape. The hymenium covered one side almost to the base, as well as the rounded top of the fructification (FIG. 44). A subhymenial layer of fine hyphae, similar to the cortical layer previously described but not so regularly delimited, was present, while the hyphae of the medulla were still large and undifferentiated. In a region in the middle and toward the base of the sporophore, however, these medullary hyphae had arranged themselves in rows which were somewhat parallel. This condition existed down through the base of the fruiting body, where the hyphae became continuous with the mycelium in the ray. This was the first evidence of the differentiation of a stalk. The cylindrical cortical cells, which are peculiar to this species, may also be seen.

In later development, the sporophore was taller and broader, but much compressed. The hymenium did not extend quite to the top of the fructification, and reached downward to a point only slightly above the top of the stalk. Growth seemed to be an intercalary expansion of the sterile hyphae, in such a fashion that the hymenium which formerly had covered much of the surface, was now confined to a single region. Development seemed to be at the expense of the compactness of the medullary hyphae, which became less dense, spreading so much in some cases that there appeared a hollow region in the middle of the sporophore. The elongation of the stipe seemed to take place beneath the lower limit of the hymenium.

When the structure is viewed in cross section, the hymenium is seen to extend about half way around the fructification. On each side, the basidia blend gradually with the cortical hairs, becoming fewer and more scattered. The hymenium is underlaid by a rather firmly woven subhymenial layer which does not seem to become thinner with the expansion of the sporophore. The basidia are small, at first cylindrical, and borne at the ends of branching hyphae. Two or three basidia in different stages of

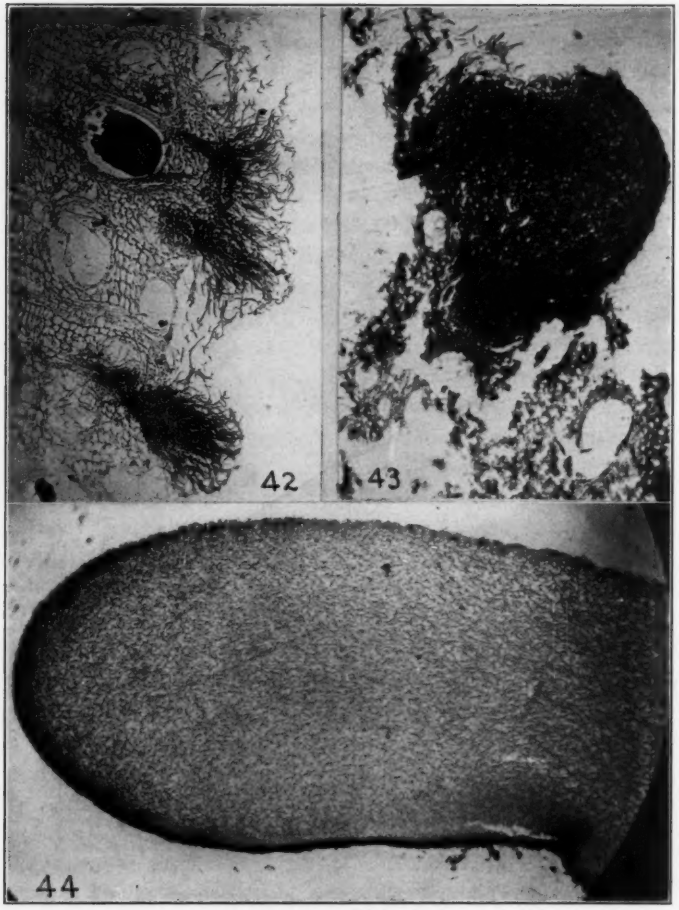

FIG. 42-44.

development may be seen upon one hyphal strand. There are no cystidia nor paraphyses. Any paraphysis-like structures which may be present are merely young basidia. No imperfect reproductive structures, such as oidia, were observed.

The basidium is a typical dacryomycetaceous basidium. The probasidium is slender and cylindrical, becoming somewhat clavate

at the time of the differentiation of the epibasidia. The two epibasidia appear at either side of the tip of the basidium and grow toward the surface of the gelatinous sheath. They have about the same diameter as the hypobasidium, and the greater number are about the same length. Some, however, which are very close to the surface may have very short, stubby epibasidia, and a few which are deeply embedded may have longer, thinner ones.

The spores in the material used in this study measured 9–10 × 3–4 μ. These measurements agree most closely with Fisher's, but are well within the limits given by Burt and others. They are apiculate, allantoid and are one-celled at discharge. Creager states that this is to be regarded as the mature stage, and that the appearance of the septum indicates the beginning of germination. No spore which had germinated beyond the production of a septum was observed in my material, but Creager has covered very adequately this phase of development. He found that the method of germination depends upon the type of substrate, and discovered that either a germ tube, or globose conidia, or both, may be produced. If both are produced, then each cell may produce either or both.

The young basidia are filled with a finely granular, homogeneous, rather darkly-stained cytoplasm, and contain two very small nuclei, longitudinally arranged in the middle of the basidium. These prefusion nuclei are so small that an accurate account of their structure is impossible. A careful observer, however, may note the very black, minute nucleolus surrounded by a distinct hyaline area, and a very delicate nuclear membrane (FIG. 1). There is no doubt a septum at the base of the larger basidium shown in this figure, but its presence is not brought out by the stain, as mentioned before.

The fusion of these two nuclei produces one which is from three to five times the diameter of the primary resting nuclei (FIG. 2). There seems to be no definite time at which nuclear fusion takes place. I have seen the large, distinct spindle which appears at the first division of the fusion nucleus in basidia as yet so slender that the walls were bulged to accommodate it (FIG. 5). I have also seen the same stage in basidia which were clavate, or already broadened and flattened at the tip, and even in some in which the epibasidia had appeared (FIG. 9, 10).

It seems possible that fusion initiates in the basidium the series of morphological changes that are attendant upon the maturation of the nuclei, and that the two proceed simultaneously; meiosis at some times running slightly ahead, and at others the morphological development gaining the ascendency.

The primary nuclei unite completely, and the fusion nucleus assumes the appearance of the resting stage. The single nucleolus is large and centrally placed, and the hyaline area and the nuclear membrane are very distinct. The cytoplasm retains its homogeneous appearance, apparently not becoming less dense as the basidium enlarges.

The nucleolus now disappears, and a mass of darkly-stained particles which I regard as the chromosomes appears. These at first lie loosely within the nucleus (FIG. 3), and then arrange themselves rather irregularly along the equator of the spindle (FIG. 4). The nucleus meanwhile has enlarged and become slightly oval in shape, with its long axis parallel with the long axis of the basidium. The clearly delimited hyaline area and the nuclear membrane persist. The chromosomes now begin to separate, and to move toward opposite sides of the membrane, and the strands of the spindle may be seen between the separating masses of chromatin (FIG. 5). The spindle is always parallel with the long axis of the basidium. There would be no room for any other arrangement, as the nucleus fills the basidium from wall to wall. The ends of the spindle do not converge, so that the fibers appear to be parallel (FIG. 9, 10). The chromatin material lies loosely appressed to the nuclear membrane, at opposite sides of the nucleus. The membrane, and then the spindle soon disappear and the chromosomes lie free in the cytoplasm, with a clear area between them (FIG. 7). They seem to lie almost in a straight line, side by side, and then resolve themselves into an irregular clump, which becomes smaller. A nuclear wall appears around each mass of chromosomes, the chromatin becomes reticulate in appearance, and a nucleolus appears in each daughter nucleus (FIG. 11). There is also visible in some nuclei a faint strand of darkly-staining material, connecting the nucleolus with the membrane (FIG. 12). No centrosomes or other bodies of such nature have been observed.

There appears to be a tendency on the part of the nuclei to migrate to the tip of the basidium, and in many cases one of the two large, darkly-stained resting nuclei may be seen close to the extreme tip (FIG. 11, 14). It is quite probable that this is due to the fact that in the enlarging basidium the cytoplasm tends to move toward the tip, carrying the nuclei with it. A second division now follows, and four nuclei may be seen, arranged longitudinally in the probasidium (FIG. 15).

The morphological development proceeds as follows: The basidium becomes longer and thicker, but remains very symmetrical in shape, and the cytoplasm continues to be very dense. At the same time, the primary nuclei fuse, and then proceed to the first of the meiotic divisions. Before there is any further change in the basidium, the daughter nuclei may divide, so that four nuclei are to be found, lying in a row, well up toward the tip of the basidium, as mentioned above. In other cases, the development of the basidium proceeds more rapidly, and by the time the first division has been completed the tip of the basidium has grown broad and flat and the two epibasidia have appeared (FIG. 13). Occasionally, well-developed epibasidia were seen while the fusion nucleus was still in the metaphase of the first division (FIG. 10).

When the epibasidia have become about one-fourth the length of the hypobasidium, the cytoplasm of the latter begins to appear noticeably paler and thinner, and conspicuous vacuoles may be seen at the base. At this stage, four nuclei are usually apparent. The two nuclei which are nearest the tip are the first to pass into the epibasidia. They move up toward the top of the hypobasidium until the uppermost one seems almost to come in contact with the wall at the tip. This nucleus then moves into one of the epibasidia, while the second, after also passing up directly to the tip, enters the other epibasidium (FIGS. 16, 17). The second pair of nuclei now come up to the top of the hypobasidium, separate as before, and also pass, one into each of the epibasidia (FIG. 18, 19). There are some variations. On one occasion, the first two nuclei had already passed into the epibasidium, while the second daughter nucleus, as yet undivided, lay almost at the tip of the hypobasidium (FIG. 22). In many cases, the first two nuclei passed into one epibasidium, the other two into the second (FIG.

20, 21). In another, one epibasidium was aborted, and all four nuclei passed into the second, which produced a spore containing at maturity only one nucleus (FIG. 23). The indications are, however, that in the larger number of cases, two nuclei pass into each epibasidium.

As the epibasidia approach the surface they become narrower and a sterigma develops at the tip of each (FIG. 17). They grow through the gelatinous surface of the hymenial layer to the outside, and then upon the sterigma there appears a slight swelling or vesicle which grows larger, and fills with protoplasm (FIG. 19). Sometimes a nucleus has by this time reached the tip of the epibasidium and passes into the spore while this spore is as yet very small. At other times the spore, although almost mature in size, may contain no nucleus, and two nuclei may be seen in the epibasidium (FIG. 27).

The moving stream of cytoplasm carries the nucleus to the extreme end of the spore, which is so placed upon the sterigma that the concave surface is toward the axis of the basidium (FIG. 28). The sterigmata are long enough to permit the spore to be discharged without encountering any obstacle.

The spores are so large in proportion to the size of the basidia that by the time they are mature there is little cytoplasm left in the basidia, which appear to be completely collapsed. In many instances, the two spores seem to appear and to mature simultaneously. The cytoplasmic material is then divided equally between them. At other times the development of one spore exceeds that of the other, and in such cases, E. J. Gilbert's conjecture (**9**), in the case of the Boletaceae, that the tardiness of the second spore deprives it of its cytoplasm and of its opportunity for development seems highly probable.

The spores are uninucleate. After they are discharged from the sterigmata, the nucleus moves from the distal end, where it has been forced by the entering cytoplasm, and takes up a position in the middle of the spore. Here it divides once, and the two daughter nuclei move away from the middle of the spore. A septum is then laid down at right angles to the long axis, and the spore is ready for germination. Killermann (**13**) and Teng (**19**) mention a three-celled spore, and Creager a four, but none such have been observed in this study.

The second nucleus remains in the epibasidium or hypobasidium. The epibasidium has been observed bearing a completely mature spore containing one nucleus. The second nucleus was visible in the already partially collapsed epibasidium, apparently held in place by the deflated walls, which seemed too narrow to permit it to pass. Above this nucleus the epibasidium had collapsed; below, the presence of a small amount of protoplasm slightly distended it. The line of separation was very distinct (FIG. 26). A complete basidium in which one nucleus had remained in the hypobasidium was also found. Faint strands of cytoplasm held it in place, while below it the empty walls were beginning to collapse. One of the epibasidia still held a deeply stained, uninucleate spore, and the other, from which the spore had been discharged, contained another nucleus, also held in place by cytoplasmic strands (FIG. 25).

The movement of the cytoplasm through the basidium, and its entrance into the spore seems to be a physical process, entirely subject to the variations which might prevail in protoplasmic movement directed toward a certain point by the release of pressure brought on by the expansion of a cell membrane. If the basidium grows rapidly, the protoplasm may flow quickly to the tip, carrying the nuclei with it. When growth is slower, the cytoplasm will move more slowly, and one or more of the nuclei, which seem to be heavier than the cytoplasm, may be left behind. If the nuclei are close to the wall, they may also be held there. One epibasidium may grow more rapidly than the other, in which event, two successive nuclei will pass into one; but if the two grow at the same rate, then one nucleus may pass into one, the other into the second. The reason why the third and fourth nuclei do not pass into the spores is not apparent, but the evidence seems clear that this does not occur.

The production of two sets of spores seem improbable. The spores are so large in proportion to the size of the basidium that after two are produced, the basidium is almost entirely depleted. Buller describes the collapse of the basidium after the discharge of the spores in other members of the Dacryomycetaceae, and E. J. Gilbert brings forward the same argument for the Boletaceae. He also cites the discovery of "giant" spores upon basidia which matured only one sterigma as a proof of the same statement.

The nuclei are so small that a count of the chromosomes is almost impossible. There are certainly more than two. When the chromosomes separate at the anaphase, they are grouped together so closely that there may appear to be only two, but a more detailed examination shows that several discrete particles are present in each group.

SUMMARY

1. The mycelium of *Guepinia Spathularia* fills medullary rays and vessels of decaying wood, giving rise to fructifications at weak places where branches break through to the surface.

2. Differentiation begins in the cortex. This is followed by the formation of a hymenium, and later, of a stalk. Finally, some changes take place in the appearance of the medulla. The hyphae are binucleate, but no clamp connections have been found.

3. The young basidium is paraphysis-like, and binucleate. Its enlargement is inaugurated by the fusion of the primary nuclei.

4. Two divisions of the fusion nucleus give four nuclei, longitudinally arranged in the basidium. The first of these divisions is regarded as the reduction division, but the chromosome number was not determined.

5. Two nuclei pass into each epibasidium, but only one passes into each spore. The other remains in the epibasidium, there degenerating.

6. The spores are uninucleate at discharge, but the nucleus soon divides and a septum is laid down, making the mature spore two-celled.

The work was done in the mycological laboratory of the State University of Iowa, under the direction of Professor G. W. Martin. Photomicrographs were made by Mr. Travis W. Brasfield.

THE IMMACULATA,
CHICAGO, ILLINOIS

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EXPLANATION OF FIGURES

All figures showing microscopic detail drawn with aid of camera lucida, using Zeiss apochromatic objective HI90 and compensating ocular K15 and reduced in reproduction to approximately × 1000.

FIG. 1, hyphal tip showing two young basidia, the two lower nuclei about to pass into the smaller basidium; 2, basidium, showing fusion nucleus; 3, prophase of first division of fusion nucleus; 4, metaphase; 5, 6, late anaphase, nuclear wall and spindle shown; 7, late anaphase, nuclear wall and spindle have disappeared; 8, fusion nucleus in developing basidium; 9, basidium in about the same stage as preceding, late anaphase; 10, anaphase in basidium with well-developed epibasidia; 11, binucleate stage; 12, same stage as preceding, lower nucleus showing attachment between nucleolus and nuclear membrane; 13, binucleate stage in well-developed basidium; 14, prophase of second division; 15, four nucleate stage; 16, first nucleus has passed into epibasidium, second almost touching tip of hypobasidium; 17,

18, one nucleus has passed into each epibasidium, sterigmata developing; 19, spore-vesicles appearing at tips of sterigmata; 20, 21, two upper nuclei apparently entering same epibasidium; 22, two nuclei in one epibasidium, undivided nucleus about to pass into second; 23, one nucleus in spore, three in same epibasidium; 24, mature spores not yet discharged; 25, degenerating basidium with mature spore attached, the other presumably discharged; two nuclei and traces of cytoplasm left in basidium; 26, degenerating epibasidium containing one nucleus and bearing a mature spore; 27, developing spore-vesicle with two nuclei in epibasidium; at right, younger spore already containing a nucleus; 28, mature spore, still attached to sterigma; 29, spore as discharged from sterigma; 30–35, stages in division of spore nuclei; note difference in orientation of spindle in 31 and 32; 36, 37, first stages in germination; the empty cell in 36 shows plainly the presence of a septum; 38, longitudinal section of young sporocarps growing upon decayed wood: *a*, transverse growth of mycelium through medullary rays, *b*, longitudinal growth through vessels, *c*, growth of mycelium from rays into vessels, *d*, fanlike spreading of hyphae at surface; 39, formation of young sporocarp within a vessel, the mycelium entering the vessel through a ray; note absence of covering of loose hyphae; 40, origin of sporophore from mycelium in medullary ray, showing root-like thickening in the ray and globose head of the fructification; the mycelium of the head has become compact and the tomentose covering has begun to disappear; 41, more mature stage; the larger hyphae of the medulla, the finer cortical layer and the rind or cortex may be seen; 42, sporophores developing from rays; fructification above shows a compact basal portion which will form the body and a loose tomentum which will disappear; below an older stage of a similar sporophore; 43, sporophore which has developed from a trachea; the portion of the cortex which is shown is folded back upon the medulla; 44, young sporophore with hymenium already mature; the shape is no longer globose; elongation has begun; originally erect, the hymenium was at one side, passing part way over the top; on opposite side (toward top of page in illustration) may be seen the sterile cortical layer; the hyphae at the base are parallel in anticipation of the formation of the stalk.

EDITOR'S NOTE

There is a difference of opinion on the spelling of the generic name *Dacryomyces*. The original spelling was with the "o." When Fries took up the name from Nees he spelled it without the "o." Whether intentionally or accidentally, we can only conjecture. The editor believes that it was purely an error on the part of Fries, and since the original spelling has been used in the recent volumes of MYCOLOGIA, it is retained here as an editorial policy and in the interests of consistency.

A MONOGRAPH OF THE GENUS CUNNINGHAMELLA WITH ADDITIONAL DESCRIPTIONS OF SEVERAL COMMON SPECIES

GORDON D. ALCORN AND CHARLES C. YEAGER

(WITH 2 FIGURES)

The genus *Cunninghamella* is an interesting one showing an unusually fine turf, well defined conidiophores and conidia, and rapid growth. Its history, though not a long one, is absorbing to the mycologist. As many of our present forms, the species *C. echinulata* Thaxter, was, in 1891, first placed in the Fungi Imperfecti as *Oedocephalum echinulatum*. In 1903, Matruchot transferred this species to the Mucorales on the basis of its nonseptate mycelium, and because of its liability to be attacked by *Piptocephalis*, an obligate parasite on the Mucorales and a member of the Cephalidaceae. The perfect stage, zygospores, was not known until 1904 when their discovery by Blakeslee conformed the previous, unusual diagnosis. Since that time the remaining species have been presented, some of which have not shown zygospores, but because of outstanding similarities, must be placed with preceding forms of this genus.

Due to the difficulty in identifying the various species of the genus under discussion, because of the scattered references, the authors felt that a bringing together of the known species as well as a list of the literature describing them, might prove helpful to students of Mycology. This problem of identification was forcibly brought to our attention, during the last year and a half, with the discovery as soil forms and laboratory contaminants, several species not agreeing with published descriptions.

Our cultures of *C. elegans* and *C. Bertholletiae* were so different from original descriptions that difficulty in identification was experienced. Several of our soil isolations were sent to Carnegie Institution at Cold Spring Harbor. Here Dr. Blakeslee and Miss

Satina made contrasts with our various cultures as well as known tester strains. One proved to be a (+) strain of *C. elegans,* and the other a (+) strain of *C. Bertholletiae* because of production of zygospores with Dr. Blakeslee's known (—) strains of these species.

It was thought that the key would be useful where original descriptions were not available. Also it was deemed advisable to insert our own descriptions following various species whose original descriptions seemed to be inadequate.

The authors wish to thank Miss Satina, and Dr. Blakeslee of Cold Spring Harbor, Mr. Lynn Aitken of Kansas State Agricultural College, and Mr. Louis K. Mann, University of Idaho, who assisted in the investigation and photography; also Miss Ellen D. Chandler, University of Idaho, who aided in the translation of the original articles, and offered helpful suggestions in the preparation of the added descriptions.

KEY TO SPECIES

A. Terminal vesicles more than 50 μ in diameter.
B. Lateral branches irregularly—cymosely branched.
C. Turf white; terminal conidia oval, 9–18 μ wide, 12 μ long, long-echinulate, pedicillate.....1. *C. africana.*
CC. Turf white becoming yellowish; terminal conidia rounded, 7–10 μ in diameter.....2. *C. albida.*
BB. Lateral branches opposite or whorled, variable in number.
D. Turf white to ashy; conidiophores dichotomous; lateral branches more than 30 μ long.....3. *C. elegans.*
DD. Turf white to silvery; conidiophores not dichotomous; lateral branches less than 30 μ long.....4. *C. verticillata.*
AA. Terminal vesicles less than 50 μ in diameter.
E. Conidiophores unbranched.....5. *C. microspora.*
EE. Conidiophores branched.
F. Terminal vesicle rounded, over 30 μ in diameter.
G. Lateral conidia smaller than terminal..6. *C. Bertholletiae.*
GG. All conidia similar.....7. *C. echinulata.*
FF. Terminal vesicles round-truncate, under 30 μ in diameter. 8. *C. Blakesleana.*

DESCRIPTION OF SPECIES

1. CUNNINGHAMELLA AFRICANA Matruchot.

Turf white, filaments interwoven; conidiophores erect, aseptate; terminal vesicles spherical, 50–100 μ in diameter; lateral branches irregularly to cymosely arranged; lateral vesicles similar to terminal; terminal conidia oval, 9–18 μ wide by 12 μ long, pedicillate, long-echinulate; lateral conidia similar to terminal.

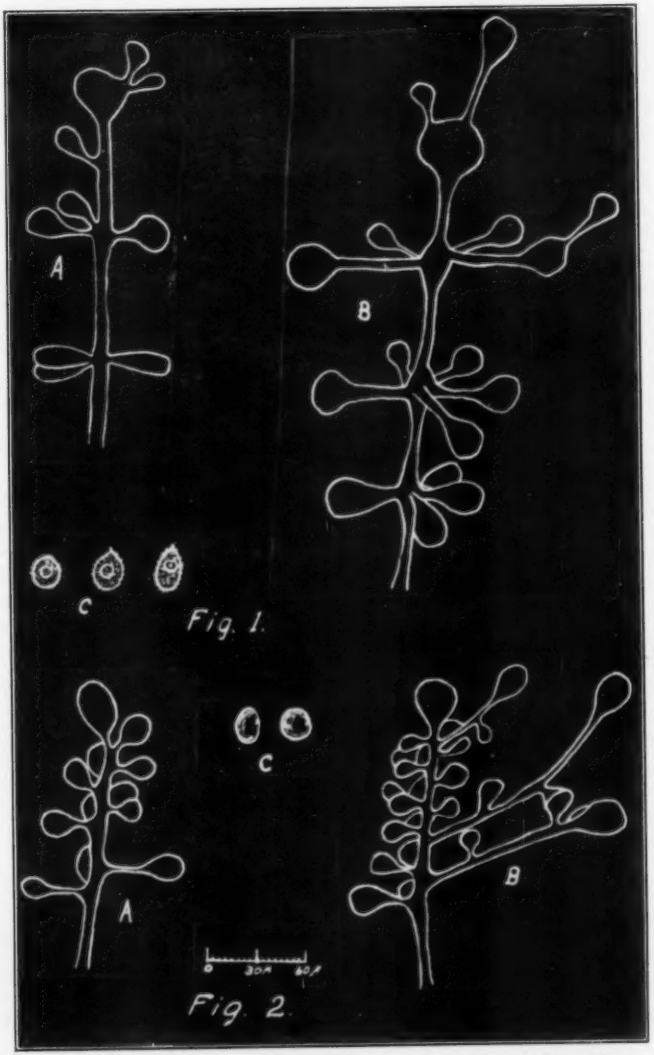

FIG. 1. *Cunninghamella elegans.* a, b, showing normal branching; c, spores 3 × the scale. 2, *Cunninghamella Bertholletiae.* a, b, detail of branching; c, spores 3 × the scale.

2. CUNNINGHAMELLA ALBIDA (Sacc.) Matruchot.

Turf white to yellowish, interwoven, aseptate; conidiophores erect, often sub-fasciculate, usually not branched; terminal vesicles spherical, not more than 100 μ in diameter; lateral branches if present similar to those of *C. africana;* lateral vesicles similar to terminal, but slightly smaller; terminal conidia 7–10 μ in diameter; lateral conidia slightly smaller.

3. CUNNINGHAMELLA ELEGANS Lendner.

Turf white to ashy, filaments firm and interwoven; conidiophores erect, dichotomous, aseptate; terminal vesicles inflated, regular, round to oval, 40–60 μ in diameter; lateral branches whorled, variable in number, lateral vesicles 18–20 μ in diameter, round; terminal conidia ovoid to pyriform, 16–22 μ long by 12–14 μ wide, short-echinulate; lateral conidia spherical, 8–10 μ in diameter.

Our description is as follows: Turf white to silver, spreading; filaments firm and interwoven, 7–13 μ wide, with abundance of oil; circinate portions typical; conidiophores erect, multi-branched; terminal vesicles 27–35 μ in diameter, spherical, smooth; lateral branches lacking or up to 3 whorled, place of attachment to conidiophore swollen; sub-terminal whorl 38 μ long, vesicles spherical, 16–28 μ in diameter; smooth; intermediate whorl 24 μ long, vesicles spherical 14–16 μ in diameter, smooth; basal whorl of pyriform branches, 14 μ wide by 26 μ in length, smooth; superbranches, arising from terminal head, of varying lengths; vesicles spherical; terminal conidia lemon shaped, bearing sterigmata after separation from vesicle, 12 μ long by 9 μ in width, very finely echinulate; lateral conidia ovate in varying degrees; 6 μ wide by 10 μ in length; asterigmatate, very finely echinulate. Isolated as soil form in Moscow, Idaho, 1937.

4. CUNNINGHAMELLA VERTICILLATA Paine.

Turf white to silvery, loose, erect, 2–4 cm. in height; conidiophores long, 2 cm. or more, aseptate; terminal vesicles globose to oval, about 50 μ in diameter; lateral branches numerous, not exceeding 30 μ long, subterminal, whorled, the conidiophores swollen at point of attachment of lateral branches; lateral vesicles pyriform to oval, not over 16 μ in diameter; terminal conidia ellipsoid, pointed at the attached end, 10 μ by 13–15 μ, very finely echinulate; lateral conidia oval, bluntly pointed at the attached end, 8–12 μ in diameter, very finely echinulate.

5. CUNNINGHAMELLA MICROSPORA (Rivolta) Matruchot.

Turf white, small, interwoven; conidiophores erect, unbranched; terminal vesicles rounded, minutely papillate, about 20 μ in diameter; conidia obovate, basally subapiculate, finely papillate, colorless, less than 7 μ wide.

6. CUNNINGHAMELLA BERTHOLLETIAE Stadel.

Turf white to light-olive, filaments firm and interwoven; conidiophores erect, branched, aseptate, abundantly supplied with oil; terminal vesicles rounded, 30–40 μ in diameter; lateral branches irregularly arranged; lateral vesicles similar to terminal; terminal conidia ovoid, 8–12 μ in diameter; lateral conidia similar but smaller.

Our description is as follows: Turf gray, filaments firm and interwoven; conidiophores erect, irregularly-cymosely branched, aseptate; terminal vesicles ovate, about 25 μ wide by 33 μ long; lateral branches variable, alternately arranged in groups, numerous, 22–55 μ long; lateral vesicles round, about 23 μ in diameter; terminal conidia ovate, smooth, 5 by 9 μ; lateral conidia similar to terminal but slightly smaller. Isolated as soil form in Moscow, Idaho, 1937.

7. CUNNINGHAMELLA ECHINULATA Thaxter.

Turf white becoming yellowish with age; filaments interwoven; conidiophores erect, more or less irregularly and indefinitely branched; terminal vesicles very variable in size, areolate, nearly spherical to obovoid, maximum 45 μ by 65 μ, average 28 μ by 35 μ; lateral branches similar to terminal but smaller; all conidia oval to elliptical; finely echinulate; average 10 μ by 12 μ, maximum 18 μ by 25 μ.

8. CUNNINGHAMELLA BLAKESLEANA Lendner.

Turf white to ashy, filaments densely interwoven; conidiophores erect, branched; terminal vesicles rounded, slightly truncate, minutely verrucose, 30 μ by 28 μ; lateral branches alternate, variable in number; lateral vesicles somewhat smaller than terminal, round; terminal conidia ellipsoid, 13–15 μ by 9–12 μ, finely echinulate; lateral conidia spherical, colorless, 10 μ in diameter. Isolated as an Agar contaminant in Moscow, Idaho, 1937.

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PHOTOGRAPHS AND DESCRIPTIONS OF CUP-FUNGI—XXX. ARACHNOPEZIZA [1]

FRED J. SEAVER

(WITH 1 FIGURE)

The above named genus was established by Fuckel with *Peziza aurelia* Pers. as the type. While this fungus is one of the more minute forms it never fails to excite the interest of the collector because of the beautiful golden or pale-orange apothecia seated on the spiderweb-like subiculum, which has suggested the name *Arachnopeziza,* the specific name referring to the color.

This species has been rather frequently encountered by the writer although it can scarcely be said to be common. It has also occasionally been sent in for determination. It occurs on decaying leaves and twigs, and very often on old acorn cups. Just why it should show a preference for the latter is difficult to say. The outside of the minute apothecia is clothed with long slender hairs which often stand up in agglutinated tufts resembling minute teeth about the margin. These become strongly incurved in dried specimens, concealing the hymenium. The species is characterized by its fusoid spores which become 3-septate at maturity and often with a bristle-like apiculus at either end.

The photograph accompanying this article was made from material collected by Dr. J. F. Adams at Pennsylvania State College in 1917. The author was having some difficulty in getting mature spores for these illustrations. During the process the work was left long enough to go to the mail box when to his surprise a specimen of the same species was sent in for determination by Maurice B. Walters from Cleveland, Ohio. This fresh material supplied just the characters which were needed to complete the drawing. It is an interesting coincidence that this species, which is rarely sent in, should have come just at this critical moment.

[1] This paper is preliminary to a monograph of North American Cup-fungi (inoperculates), a companion volume to North American Cup-fungi (operculates), which was published by the author and issued in December, 1928.

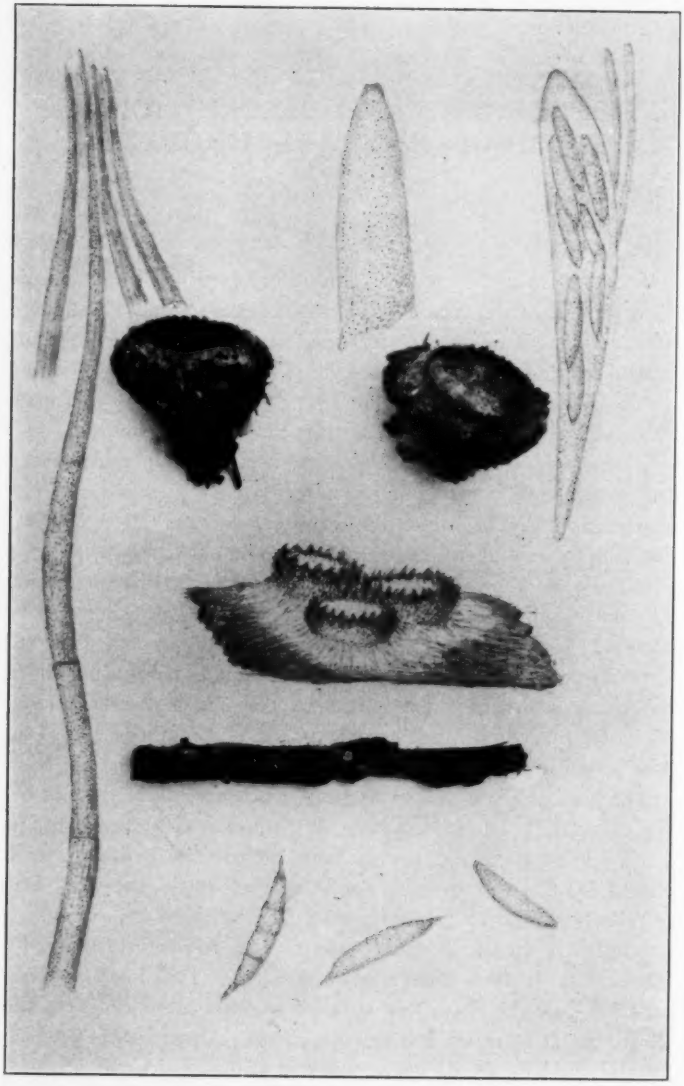

FIG. 1. *Arachnopeziza aurelia.*

We have in the collection in The New York Botanical Garden another species which is usually placed in this genus, *Arachnopeziza aurata* Fuckel. This material was collected by A. P. Morgan at Preston, Ohio, and determined by George Massee of England. This species is characterized by the long filiform spores. Also a third species sent by Dr. Bisby from Canada seems to conform to the description of *Arachnopeziza delicatula* Fuckel. All of these species have at one time or another been placed in the genus *Tapesia,* although the two genera were separated by Fuckel. For the time being at least the genus *Arachnopeziza* will be kept distinct. Additional material is solicited. Following is the diagnosis of the genus and species:

ARACHNOPEZIZA Fuckel, Symb. Myc. 303. 1849.

Apothecia gregarious, seated on a thin spiderweb-like white or yellowish mycelial subiculum, at first closed and rounded, opening and becoming patellate or scutellate, externally clothed with fine bristly hairs; asci clavate, 8-spored; spores ellipsoid to fusoid clavate or filiform, becoming several-septate and often with an apiculus at each end, hyaline; paraphyses filiform, usually enlarged above.

Type species, *Peziza aurelia* Pers.

Spores, fusoid, apiculate 4–5 × 15–20 μ.....1. *A. aurelia.*
Spores clavate or filiform.
Spores clavate, 3.5 × 40 μ.....2. *A. delicatula.*
Spores filiform, 2.5–3 × 65–75 μ.....3. *A. aurata.*

1. ARACHNOPEZIZA AURELIA (Pers.) Fuckel, Symb. Myc. 303. 1849.

Peziza aurelia Pers. Myc. Eur. **1**: 270. 1822.
Peziza Wauchii Grev. Scot. Crypt. Fl. *pl. 139.* 1825.
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Belonidium aurelia DeNot. Comm. Soc. Critt. Ital. **1**: 381. 1863.
Patellaria bicolor Curr. Trans. Linn. Soc. **24**: 491. 1864.
Polynema aurelium Fuckel, Symb. Myc. Nachtr. **1**: 49. 1871.
Lachnella aurelia Quél. Enchir. Fung. 315. 1886.
Tapesia fulgens Hazsl. Zool.-Bot. Verh. 163. 1887.

Tapesia candidofulva Sacc. Syll. Fung. **8**: 385. 1889.

Belonidium fulgens Sacc. Syll. Fung. **8**: 500. 1889.

Apothecia gregarious, seated on spreading white or yellowish mycelial web, sessile, at first rounded then becoming scutellate externally golden-yellow to pale-orange, clothed with fine hairs, reaching a diameter of 2–3 mm.; hymenium yellowish, a little paler than the outside of the apothecium; hairs slender, septate, reaching a length of 100 μ and a diameter of 2 μ, tapering to a slender point, collected into conical tufts which stand up about the margin like teeth; asci clavate, attenuated above, reaching a length of 70–90 μ and a diameter of 8–10 μ; 8-spored; spores fusoid, hyaline, becoming 3-septate, 4–5 $\times$ 15–20 μ, often with an apiculus at either end; paraphyses filiform, slightly enlarged above.

On leaves, soil, twigs and acorn cups.

TYPE LOCALITY: Europe.

DISTRIBUTION: New York to Pennsylvania, Iowa and Manitoba; also in Europe.

ILLUSTRATIONS: Fuckel, Symb. Myc. Nachtr. **1**: *f. 35;* Scot. Crypt.-Fl. *pl. 139;* Trans. Linn. Soc. **24**: *pl. 51, f. 15–16;* Boud. Ic. Myc. *pl. 520.*

2. ARACHNOPEZIZA DELICATULA Fuckel, Symb. Myc. 304. 1869.

Belonidium delicatulum Sacc. Syll. Fung. **8**: 499. 1889.

Apothecia gregarious or scattered, seated on a delicate, white arachnoid subiculum, at first globose and closed finally expanding, reaching a diameter of 1–2 mm.; hymenium concave, reddish-brown; asci clavate-cylindric, reaching a length of 80–100 μ and a diameter of 8–10 μ; 8-spored; spores elongated, clavate, slightly curved, simple or becoming sparingly septate, reaching a length of 40 μ and a diameter of 3.5–4 μ; paraphyses filiform slightly enlarged above, reaching a diameter of 3 μ.

On wood and bark.

TYPE LOCALITY: Europe.

DISTRIBUTION: Quebec; also in Europe.

3. ARACHNOPEZIZA AURATA Fuckel, Symb. Myc. 304. 1870.

Belonidium auratum Sacc. Michelia **1**: 66. 1879.

Tapesia aurata Massee, Brit. Fungus-Fl. **4**: 299. 1895.

Apothecia gregarious, sessile, at first closed, then expanding, externally yellowish clothed with hairs, reaching a diameter of

.5 mm. on a thin subiculum; hymenium a little darker than the outside of the apothecium; hairs long, cylindric or tapering gradually toward the ends, septate, reaching a length of 60–85 μ and a diameter of 4 μ; asci clavate, the apex somewhat pointed, reaching a length of 96 μ and a diameter of 7 μ, 8-spored; spores filiform or slightly clavate, becoming multiseptate, slightly bent, 2.5–3 $\times$ 65–75 μ; paraphyses very slender, hyaline, occasionally branched.

On wood or the inside of bark.

TYPE LOCALITY: Europe.

DISTRIBUTION: Ohio; also in Europe.

THE NEW YORK BOTANICAL GARDEN

EXPLANATION OF FIGURES

FIG. 1. Photograph of two acorn cups and a twig with drawing in the center of three apothecia of *Arachnopeziza aurelia* much enlarged. Upper right, an ascus with spores and paraphyses and an empty ascus showing rupture. Upper left, clump of hairs. Below, three spores.

NEW CALIFORNIA FUNGI [1]

DAVID H. LINDER

(WITH 10 FIGURES)

While studying a collection of miscellaneous fungi collected by Mr. L. C. Wheeler, for the most part from the Point Lobos Reservation in California, the writer encountered a number of interesting forms, among which were five new species, including one Pyrenomycete, one of the Fungi Imperfecti, two members of the Uredinales, and one of the Ustilaginales.

Among those species of interest because rarely collected or else because they represent extensions of either host or geographic range, is *Ophiocarpella tarda* (Hark.) Theiss. & Syd. [syn.: *Ophiodothis tarda* Harkness] which is known only from California and as a parasite on *Rhus diversiloba* Torr. & Gray, in the leaves of which the fungus produces characteristic large, black, angular spots which give a mottled or mosaic appearance to the leaf. Frequently a large part of the leaf becomes involved and thus the fungus under proper environmental conditions appears able to cause severe damage to the host. Another species of interest, originally described as occurring on *Juniperus virginiana* L. in South Carolina, is *Coccodothis sphaeroidea* (Cooke) Theiss. & Syd. [syn.: *Dothidea sphaeroidea* Cooke; *Dothidella sphaeroidea* (Cooke) Ellis & Ev.] which for the first time is reported from the Pacific Coast and on *Cupressus Goveniana* Gord. Finally there are two species of the Hypodermataceae which were kindly determined for the writer by Dr. Grant D. Darker: *Hypoderma pedatum* Darker and *Hypodermella limitata* Darker both of which occurred in the leaves of *Pinus radiata* Don.

The descriptions of the species that are considered to be new to science follow:

[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, No. 161.

Metasphaeria Wheeleri Linder, sp. nov. (FIG. 1 *a–d*)

Peritheciis solitariis, subcuticularis, ostiolatis, ostiolis papillatis et interdum hyphis in cuticulam hospitis crebrescentibus cinctis, 135–150 μ altitudine, 135–190 μ latitudine, subsphaericis vel nonnumquam pyriformibus; ascis parietibus incrassatis, circa 90 × 22 μ; ascosporis elongato-ellipsoideis, hyalinis, 4-septatis, ad septum medium constrictis, parietibus crassis, 18–19.5 × 5–6 μ; paraphysibus hyalinis, septatis, ascis nonnumquam superantibus.

Perithecia solitary, subcuticular, ostiolate, the ostiole papillate and occasionally fringed with mycelial outgrowths which penetrate into the cuticle of the host, 135–150 μ high, 135–190 μ broad, subsphaerical to broadly pyriform; asci thick-walled, approximately 90 × 22 μ; the ascospores elongate-ellipsoid, hyaline, 4-septate, deeply constricted at one of the median septa, 18–19.5 × 5–6 μ, thick-walled; paraphyses hyaline, septate, of irregular length, the longer ones exceeding the length of the asci.

On scales and stems of *Arceuthobium campylopodium* A. Gray, Point Lobos Reserve, California, *L. C. Wheeler*, No. *4453*. TYPE.

So far as can be determined from the literature, no species of *Metasphaeria* has been reported as occurring on *Arceuthobium* or related host genera. This species of fungus is therefore of interest in that it is a natural parasite of a parasitic host, attacking as it does the stems which it girdles, thus killing that portion of the plant which is beyond the infected area. The stems of the host become yellowish and stand out in marked contrast to the brownish-green stems of the healthy plants.

Septoria crassospora Linder, sp. nov. (FIG. 2 *a–c*)

Pycnidiis amphigenis, in maculis luteo-viridibus vel laete coloratis, subglobosis, mamillate ostiolatis, (135)–165–180 μ latitudine, (120)–135–160 μ altitudine; conidiophoris hyalinis, simplicibus, fastigatis; conidiis hyalinis vel dilute roseis, cylindricis, ad apicem rotundatis, basem fastigatis truncatisque, 24.5–37 × 3.5–5.5 μ, 3-septatis, non ad septis constrictis, parietibus et septis proportionate crassis.

Pycnidia amphigenous, in light-colored or yellowish-green areas which are of irregular size, often coalescing to occupy nearly the entire surface of the leaf, sub-globose to depressed-globose, (135)–165–180 μ in width, (120)–135–160 μ in height, ostiolate the ostiole somewhat mamillate; conidiophores simple, hyaline, tapering, one-half to one-quarter the length of the mature spores;

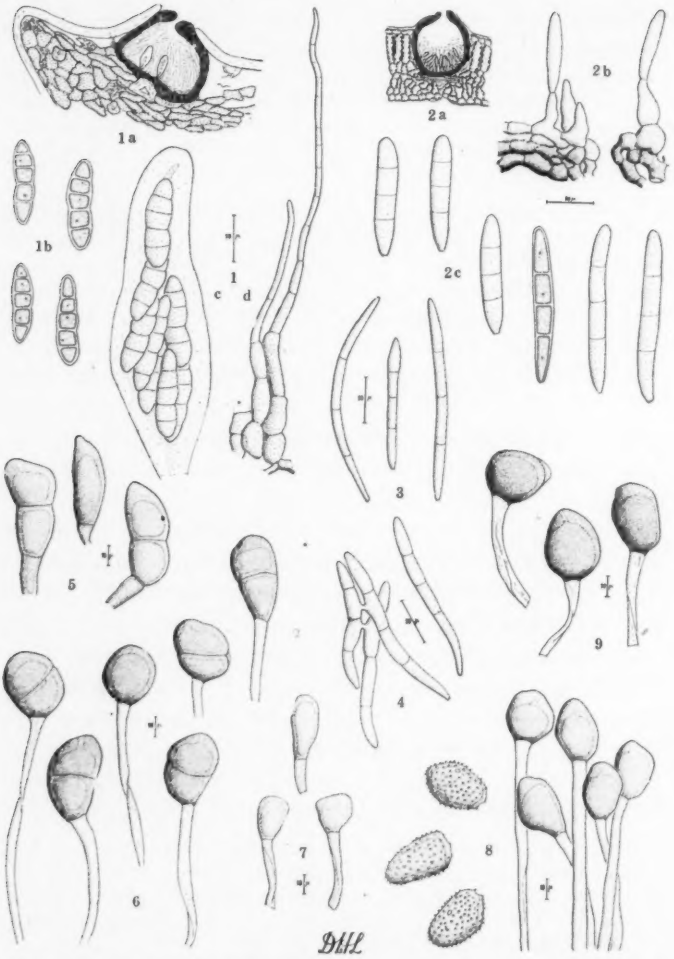

FIG. 1, *Metasphaeria Wheeleri*; 2, *Septoria crassospora*; 3, *Septoria Negundinis*; 4, *Septoria incondita*; 5, *Puccinia Sedi*; 6, *Puccinia Echeveriae*; 7, *Uromyces Galii*; 8, *Uromyces Galii-californici*; 9, *Uromyces Spermacoces.*

the conidia acrogenous, hyaline or dilutely rose-colored in mass, 24.5–37 × 3.5–5.5 μ, rounded at the apical end and tapering to the truncate basal end, cylindrical, straight or slightly curved, 3-septate, the walls and septa relatively thick.

In leaves of *Acer Negundo* L. var. *californicum* Sargent, Carmel River, Monterey Co., California, July 13, 1936, *L. C. Wheeler*, No. *4232*. TYPE.

Judging by the material available, this species of *Septoria* does considerable damage to the host and it would seem that under suitable environmental conditions it might well cause severe defoliation.

Among the sixteen species of *Septoria* that have been described as occurring on *Acer* spp., this one is clearly differentiated by its relatively short but stout spores as well as by the short, stout, phialide type of conidiophores. That this species may be compared with the others that have been described as occurring on maple, the following table of spore sizes, compiled from the literature, is presented:

On leaves:
- Spores up to 24 μ long:
  - 7–12 × 1.5–2 μ ..... *S. flavescens*
  - 13–19 × 2–3 μ ..... *S. Schirjewskii*
  - 18 × 2.5 μ ..... *S. Salliae*
  - 20–22 μ long ..... *S. acerella*
- Spores more than 24 μ long:
  - 20–40 × 1.5–2 μ ..pycnidia amphigenous ..*S. Aceris-macrophylli*
  - 25–50 × 2 μ .....pycnidia hypogenous ...*S. Negundinis*
  - 30–40 × 3 μ .....pycnidia hypogenous ...*S. incondita*
  - 30–60 × 1–1.2 μ ..pycnidia amphigenous ..*S. circinata*
  - 32–40 μ long ....pycnidia ? .....*S. acerina*
  - 40–50 × 1.5–2 μ ..pycnidia ? .....*S. saccharina*
  - 40–50 × 2–2.5 μ ..pycnidia amphigenous ..*S. apatella*
  - 40–60 × 2–3 μ ...pycnidia amphigenous ..*S. marginata*
  - 44–55 × 3 μ .....pycnidia hypogenous ...*S. Pseudoplatani*

On fruit:
- 20–25 × 1.5–2 μ ..... *S. seminalis*
- 22–44 × 1.5–2 μ ..... *S. samarae*
- 30–65 × 2–2.5 μ ..... *S. samarae-macrophylli*

In view of the fact that the spores of *S. crassospora* measure 24.5–37 × 3.3–5.5 μ, it is clear that it cannot be confused with any of the species listed above, but to illustrate this point more clearly, the spores from the type specimen of *S. Negundinis* (FIG. 3) and of *S. incondita* (FIG. 4) have for comparison been drawn at the same magnification.

Puccinia Echeveriae Linder, sp. nov. (FIG. 6)

Teleutosoris atro-brunneis, pulvinatis, plus minusve concentrice dispositis in maculis ovalis, rosco-purpureis, 3 × 2 cm. diametro; teleutosporis (34.5)–37.5–50 × 22–30.5 μ, parietibus lateralibus 2–3 μ crassis, parietibus apicalibus

(2.5)–4–7.5 μ crassis, atro-castaneis, cellulis terminalibus nonnumquam apiculatis, saepissime rotundatis; foramine cellularum basilarum prope septum; stipitibus hyalinis, persistentibus, usque 120 μ longitudine. Mesosporis subsphaericis 22–27 μ diametro.

The teleutosori amphigenous, dark-brown, pulvinatem arranged more or less concentrically on the reddish-pink colored area; the teleutospores dark chestnut-brown, (34.5)–37.5–50 × 22–30.5 μ, the lateral walls 2–3 μ thick, the apical wall (2.5)–4–7.5 μ thick, occasionally apiculate but most often bluntly rounded, not or only slightly constricted at the septum; the pore of the terminal cell apical or oblique, that of the basal cell superior at or near the septum; the stipe hyaline, persistent, up to 120 μ long. Mesospores are occasionally found and are concolorous with the teleutospores, globose to subglobose, 22–27 μ in diameter.

On *Echeveria caespitosa* (Haw.) DC., east side of Big Dome, Point Lobos Reserve, Monterey Co., California, July 23, 1936, *L. C. Wheeler,* No. *4270,* TYPE; on *Echeveria farinosa* Lindl., east side of Big Dome, Point Lobos Reserve, Monterey Co., California, July 23, 1936, *L. C. Wheeler,* No. *4271.*

Puccinia Echeveriae is quitę distinct from any described on the various genera of the Crassulaceae. *Puccinia exanthematica* MacOwen is described as producing spores which measure 24–32 × 14–19 μ, while *P. Sedi* Koern., of which the spores shown in figure 5 are drawn at the same magnification as are those of *P. Echeveriae,* are lighter colored, more elongate and with a pronounced apical swelling. Also the pedicels are conspicuously shorter than the length of the spores. In contrast to the mesospores of *P. Echeveriae* which are globose or subglobose, those of *P. Sedi* are elongate and relatively narrow.

Uromyces Galii-californici Linder, sp. nov. (FIG. 8)

Uredosoris hypophyllis, luteo-brunneis; uredosporis ellipsoideis vel nonnihil angulate-ellipsoideis, 31–34 × 25–28.5 μ, luteis, foraminis binis superioribus vel mediis, parietibus minute echinulatis, 2 μ crassis. Teleutosoris pulvinatis, atro-brunneis, hypophyllis, usque 1.5 mm. diametro; teleutosporis subsphaericis vel ovoideis, (25.5)–27–30.5 × (20)–22–25.5–(27) μ, parietibus castaneis vel atro-castaneis, foramine apicale, parietibus apicalibus incrassatis, (3.5)–6–8.5 μ crassis, parietibus lateralibus 1.5–3 μ crassis; stipitibus hyalinis vel laete coloratis, 80–95–(110) μ longitudine.

Uredosori hypophyllous, brownish-yellow; the uredospores ellipsoid or somewhat angularly ellipsoid, 31–34 × 25–28.5 μ, yellow, with two equatorial or supraequatorial pores, the walls

minutely echinulate, 2 μ thick. The teleutosori scattered, hypophyllous, pulvinate, up to 1.5 mm. in diameter, dark chestnut-brown, the teleutospores subsphaerical to ovoid, (25.5)–27–30.5 × (20)–22–25.5–(27) μ with chestnut or dark chestnut-brown colored walls which are provided with an apical pore, the terminal wall (3.5)–6–8.5 μ thick, the lateral walls 1.5–3 μ thick; the stipe hyaline or light colored, (52.5)–80–95–(110) μ long.

On *Galium californicum* H. & A., northwest slope of Whaler's Knoll, Point Lobos Reserve, Monterey Co., California, July 15, 1936, *L. C. Wheeler,* No. *4260,* TYPE.

The writer has compared this species with *Uromyces Galii* Dietel (FIG. 7) and with *U. Spermacoces* (Schw.) Curtis (FIG. 9) and from both these species the present one may be distinguished by the considerably longer pedicels. Also the spores of *U. Galii,* originally described from Japan, are smaller, proportionately more elongate, lighter colored and borne on colored pedicels; those of *U. Spermacoces* are larger, darker colored, and borne on hyaline pedicels. The characters of the three species are sufficiently distinct that it is unlikely that there will be any doubt as to their identity.

Doassansia Callitriches Jackson & Linder, sp. nov. (FIG. 10 *a–c*)

Maculis ambiguis, glomerulis sporarum in foliis immersis vel in cortice stipitis, diffundis, prominentibus, atro-brunneis, globosis vel depressis ellipsoideisque, 140–170–(240) μ diametro; sporis angulate subglobosis, 11–14 μ vel ellipsoideis 9.5–13 × 12.5–16 μ, parietibus 1 μ crassis vel minoribus, hyalinis vel leniter luteis; cellulis corticis sporis aequantibus vel leviter majoribus, usque 19.5–24 × 16.5 μ, parietibus castaneo-brunneis, 1.5 μ crassis, tenuiter interne verrucosis.

Spots not clearly defined; spore-balls in the mesophyll of the leaves or the cortex of the stems, scattered, prominent, dark-brown, globose or depressed ellipsoid, 140–170–(240) μ in diameter; spores angularly subglobose, 11–14 μ or ellipsoid and 9.5–13 × 12.5–16 μ, walls thin, 1 μ or less, colorless or slightly yellowish; the cortical cells slightly larger than the spores, up to 19.5–24 × 16.5 μ but more irregular, wall chestnut-brown, 1.5 μ thick, finely and closely internally verrucose.

On *Callitriche marginata* Torr. var. *longipedunculata* (Mor.) Jepson, Puddingstone Dam, San Jose Hills, Los Angeles, California, March 17, 1934, *L. C. Wheeler,* No. *2448,* TYPE (in University of Toronto Herb. and in Farlow Herb.).

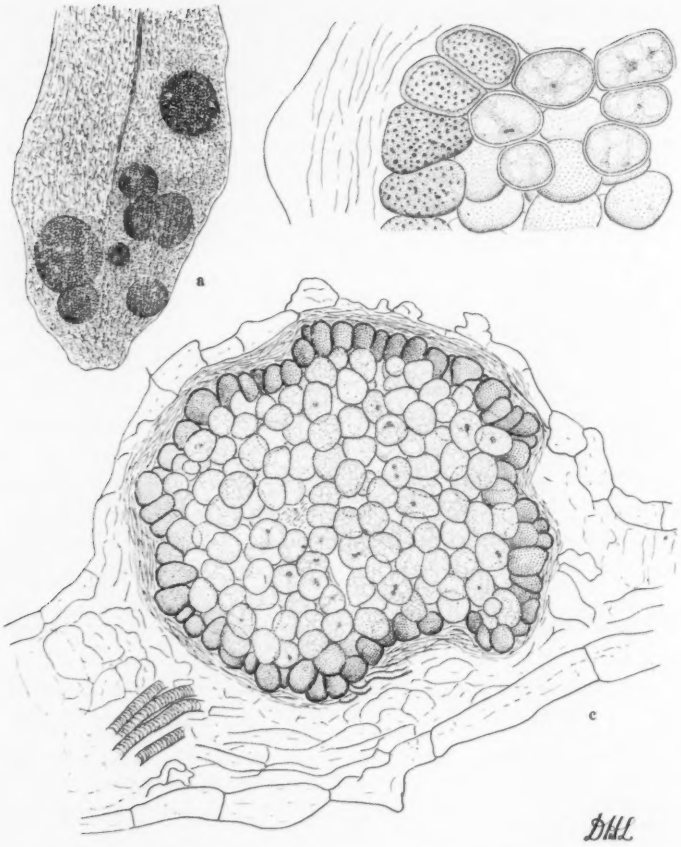

FIG. 10 a-c. *a. A* portion of the leaf of *Callitriche marginata* var. *longipedunculata* (Mor.) Jepson with the scattered sori of *Doassansia Callitriches* to show the distribution and the variation in size of the fruiting bodies in the host. Approx. 33 ×. b. A greatly enlarged portion of the margin of the sorus to show the relatively thin-walled, smooth fertile cells and the thicker walled colored sterile cortical cells which are warted internally. 1050 ×. *c.* A section through the sorus to show the relation of parasite to host. It will be noted that the sorus or spore-ball is formed in the mesophyll layer, but during the process of formation of the spore-ball the palisade layer has been crumpled and the epidermal cells have been consideraly stretched. 500 ×.

The spore-balls are larger in diameter than the normal thickness of the leaves and hence appear as prominent gall-like structures distending the tissues and covered by the epidermis. The cortical layer is differentiated by the deeper color and the thicker walls, but especially by the fact that the outer and side walls of the cortical cells are very finely verrucose on their inner surfaces, a character, easily overlooked, which has not been noted for other American species. The cortical layer, also, is not entirely made up of uniform cells since some of the outer cells are flattened and in spots appear to be formed from hyphae that are only slightly differentiated. The species is clearly differentiated from *Doassansia Ulei* Schroeter both in size of spore-balls and of spores.

EXPLANATION OF FIGURES

FIG. 1 *a–d.* **Metasphaeria Wheeleri.** *a.* Showing the subcuticular perithecium with its numerous paraphyses and two immature asci. *b.* Four ascospores which indicate the variation in size and shape, and also show the relatively thick walls and septa. *c.* A characteristic thick-walled and eight-spored ascus. *d.* Hyaline multiseptate paraphyses which are formed before ascus-formation.

FIG. 2 *a–c.* **Septoria crassospora.** *a.* Pycnidium which is formed within the palisade layer of the host leaf. *b.* Shows the origin of the conidiophores from the short-celled hyphae which make up the pseudoparenchymatous tissue of the pycnidium. Note the characteristic phialide shape of the conidiophore. *c.* Six conidia to show variation in size and shape, and one of which, drawn in optical section, shows the relatively thick walls and septa.

FIG. 3. **Septoria Negundinis** Ellis & Ev. shown here for comparison with *S. crassospora.*

FIG. 4. **Septoria incondita** Desm. of which the spores are illustrated for comparison with *S. crassospora.*

FIG. 5. **Puccinia Sedi** Koern. from specimen in Saccardo, Mycotheca Italica No. 913, and drawn for comparison with *P. Echeveriae.*

FIG. 6. **Puccinia Echeveriae** from type material on *Echeveria caespitosa,* showing variation in size and shape of spores, also showing one mesospore.

FIG. 7. **Uromyces Galii** Dietel on *Galium aparine* from Japan drawn for comparison with *Uromyces Galii-californici.* Note the short colored pedicels, and the small angular to elongate teleutospores.

FIG. 8. **Uromyces Galii-californici** from type material. The uredospores are echinulate and possess two equatorial or superequatorial pores, and the teleutospores are long pedicellate, somewhat darker colored than are those of *U. Galii* and slightly lighter colored than are those of *U. Spermacoces.*

FIG. 9. **Uromyces Spermacoces** (Schw.) Curtis from Rav. Fungi Car. *91,* on *Diodea* sp.

THE STATUS OF SEPTORIA GRAMINUM [1]

RODERICK SPRAGUE [2]

(WITH 5 FIGURES)

Septoria graminum Desm. has been recognized as a cosmopolitan and plurivorous species with slender pycnospores averaging 50–75 × 1–1.5 μ. It is reported on a wide range of hosts including wheat, oats, and a large number of field grasses (**3**, **7**, **11**). Studies by Weber on wheat (**14**, **15**) and by Sprague on oats (**13**) have shown that *S. graminum* does not occur on these cereals. Current investigations further indicate that the species is narrowly specialized and apparently distinctly limited in geographic distribution.

Much of the confusion about *S. graminum* is traceable to early studies. *S. Tritici* Rob. was described in 1842 (**4**) and issued by Desmazieres as Plantes Cryptogames de France No. 1169 (1842). The next year Desmazieres described *S. graminum* (**5**) and issued it as No. 1328 of the same series. Finally in 1848 (**6**), he placed *S. Tritici* as a variety under the later described *S. graminum*. Since the type of *S. graminum* was a meagerly described species on an unidentified grass, the status of these key species has remained unsettled since Desmazieres' paper in 1847. That the description of *S. graminum* given in Saccardo (**11**) is not based on the type but apparently on fungi studied by Berkeley, v. Thümen, Passerini, and Cooke will be pointed out in this note.

The type of *S. graminum* Desm. (Fl. Crypt. d'Fr. 1328) appears to be somewhat stunted material. The pycnidia are relatively small, dark and obscure in delimited lesions on the leaves. The

[1] Coöperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon and Washington Agricultural Experiment Stations. Published as Technical Paper No. 279 of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany.

[2] Associate Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The writer gratefully acknowledges the aid of Dr. A. G. Johnson in arranging the manuscript.

spores are small, straight, or slightly curved, non-septate or possibly up to faintly two septate. Weber (**15**) examined this type and found spores measuring 22–38 × 1 μ, and the writer, 15–42 × 0.8–1.2 μ, with a mean spore size of 25.3 × 0.9 μ. The host, which was listed as "languishing leaves of a grass," now appears to be *Brachypodium sylvaticum* (Huds.) Beauv. Most of the leaves were clipped off above their ligules, but one leaf with a ligule in the collection at Kew Herbarium, England, was determined by Mr. C. E. Hubbard, Botanist, Kew Herbarium, as typical of *B. sylvaticum*.

Much of the material assigned to *S. graminum* in herbaria, as mentioned, has much longer spores than the type. The measurements given by Saccardo (**11**), 55–75 × 1–1.3 μ, have been followed by most workers in assigning species of *Septoria* to *S. graminum*. Where Saccardo obtained these measurements is open to conjecture. Because he lists as synonyms *S. Tritici* Thüm., *S. cerealis* Pass., and *Depazea graminicola* Berk. (Ann. N. H. 103), it is believed that he assembled the species from various sources. Cooke, in early work (**2**), assigned *Sphaeria* (*Depazea*) *graminicola* Berk. to *S. graminum* Desm. without spore measurements but in later work (**3**) he used the Saccardo measurements. Berkeley assigned his *Depazea graminicola* to *S. graminum* as early as 1860 (**1**).

Since Berkeley's *Depazea graminicola* possibly represented an early collection of the long, filiform-spored species of *Septoria* on grasses an unsuccessful effort was made to locate authentic material of it. Material of his British Fungi No. *186* deposited in the Farlow Herbarium, Harvard University, proved to be a species of *Stagonospora* entirely different from a filiform *Septoria*. At the request of the writer, Miss E. M. Wakefield very kindly examined material at the Kew Herbarium of Berkeley's British Fungi No. *186* and another specimen labeled *Sphaeria graminicola* both of which occur on *Calamagrostis epigeios* (L.) Roth. She found hyaline, fusiform, 1-septate pycnospores measuring 18–20 × 2.5–3 μ, which might be *Ascochyta graminicola* Sacc. She also examined Cook's Fungi Britannica Ex. *208* on another soft leaved grass and this fungus was possibly *Scolecotrichum graminis* Fuckel. The writer is well aware of the difficulty of assembling

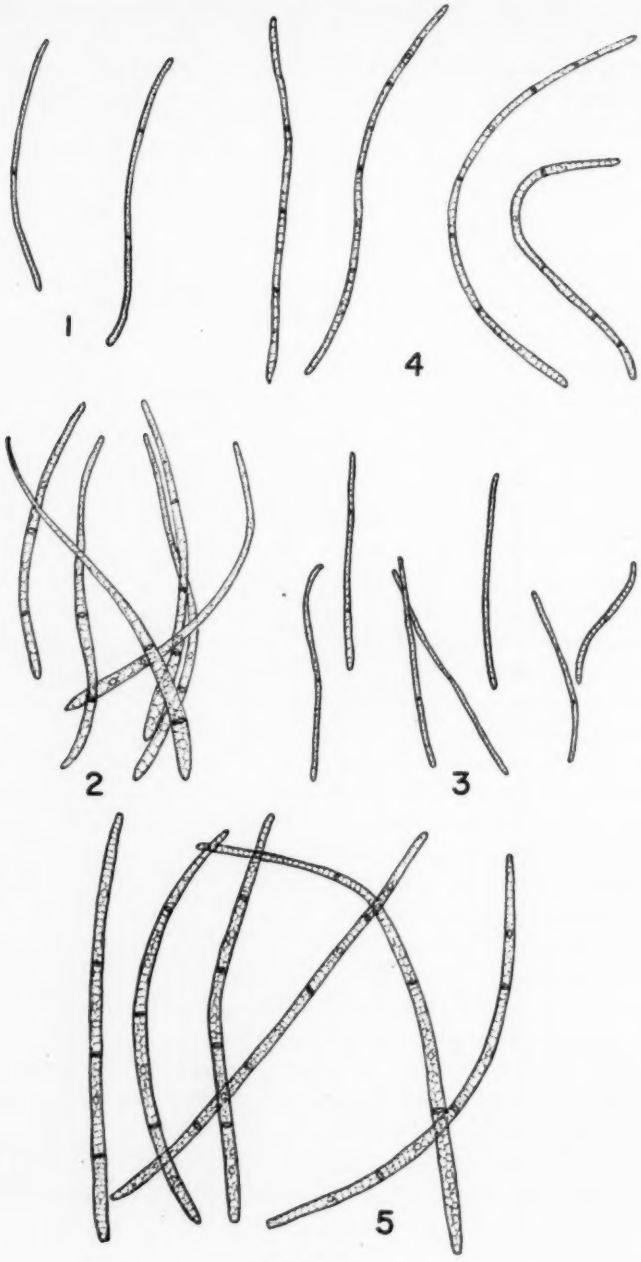

FIG. 1–5.

a collection of grass that contains only one species of fungus, and no doubt Berkeley had a filiform-spored *Septoria* in part of his collections, but it might have been destroyed in the original examinations.

Because the above listed collections of Berkeley appear to have been on *Calamagrostis epigeios,* and because the description of *Septoria Calamagrostidis* (Lib.) Sacc. (*Ascochyta Calamagrostidis* Lib.) (**12**) approaches that of *S. graminum* as listed by Saccardo (**11**) and Cooke (**2**), the writer again wrote to Miss Wakefield for type material of *Ascochyta Calamagrostidis* Lib. (**8**). Through the courtesy of Miss Wakefield and Sir Arthur Hill, Director, Royal Botanic Gardens, Kew, two slides of the type were made available for study. It was found that the fungus had very slender, curved, hyaline pycnospores borne in flattened, dark, sunken pycnidia. The spores averaged 40–55 × 0.8–1.1 μ (FIG. 1). It is clear, therefore, that Saccardo was correct in transferring the species to the genus *Septoria.* If Berkeley and Cooke saw a species of *Septoria* on *Calamagrostic,* with very slender spores, it was probably *S. Calamagrostidis* and not *S. graminum.*

There are a number of species of *Septoria* described on *Brachypodium* but some of these need not be considered as they have relatively broad spores, measuring 3–4 μ wide. *S. Bromi* Sacc. forma *Brachypodii* Sacc., however, has spores 30–40 × 1–1.2 μ and appears to be the same as *S. graminum. S. Bromi* Sacc. (FIG. 2) has filiform to narrowly obclavate, straight to somewhat curved spores measuring 45–60 × 1.2–2 μ or larger. It is evident, therefore, that *S. Bromi* forma *Brachypodii* Sacc. is distinctly different from *S. Bromi.* Vestergren made the new combination *S. Brachypodii* (Sacc.) Vestr.[3] in Sweden in 1897 (FIG. 3). *S. Brachypodii* Pass., an earlier name, has spores measuring 45–55 × 3.5 μ.

FIG. 1–5. Pycnospores of species of *Septoria*: 1, *S. Calamagrostidis* (Lib.) Sacc. from type of *Ascochyta Calamagrostidis* Lib.; 2, *S. Bromi* Sacc. on *Bromus racemosus* L., North Fork of Santiam River, Linn Co., Oregon. Ore. Herb. *10,982*; 3, *S. graminum* Desm. on *Brachypodium sylvaticum* (*S. Brachypodii* (Sacc.) Vesterg.) Vesterg. Micr. Rar. Sel *541*; 4, *S. Calamagrostidis* on *Agrostis palustris* Huds., Corvallis, Oregon. Ore. Herb. *8490*; 5, pycnospores of *S. Tritici* Rob. on *Triticum aestivum* L., Pendleton, Oregon. Ore. Herb. *10,362*. Magn. × 1,000.

[3] Vestergren. Micromycetes rariores selecti 541.

Petrak (**9**) apparently had not seen Vestergren's specimen, nor had he noted *S. Brachypodii* Pass. because he made the combination *S. Brachypodii* (Sacc.)Petrak from *S. Bromi* f. *Brachypodii*. He listed the spores as 24–42 × 1–1.5 μ. Picbauer (**10**) proposed *S. Vestergrenii* Picbauer nom. nov. after seeing Vestergren's specimen[3] and comparing it with material on *B. pinnatum* from Bulgaria.

S. graminum Desm. is believed to have medium size spores mostly 15–55 × 1–1.5 μ as indicated by the type and the apparently similar and more mature material collected by Saccardo (**11**) and Vestergren.[3] Vestergren's collection shows delimited lesions, as in the type of *S. graminum,* with small, flattened but not elongated pycnidia. *S. graminum* is close to *S. Calamagrostidis.* While it is possible that further study will indicate that it is only a variety, it appears to have distinctly shorter spores than *S. Calamagrostidis* and therefore is worthy of specific rank. The synonymy for and emended description of *S. graminum* are as follows:

S. GRAMINUM Desm. (emended)

syn. *S. Bromi* Sacc. forma *Brachypodii* Sacc.
S. Brachypodii (Sacc.) Vestr. non Pass.
S. Brachypodii (Sacc.) Petr. non Pass.
S. Vestergrenii Picbauer

Lesions on leaves linear, pale straw with narrow brown border and sometimes wider surrounding areas of pink. Pycnidia not abundant, not prominent, globose, flattened, erumpent, ostiolate, black, mostly 70–100 (40–120) μ in diameter. Pycnospores hyaline, filiform, mostly 24–45 (15–55) × 0.8–1.5 μ, aseptate to faintly one septate or sometimes two septate. The pycnospores are typically very uniformly narrow, somewhat curved and faintly septate.

On *Brachypodium* spp. in Europe.

Studies in progress in Oregon and Washington with a number of species of *Septoria* on twenty genera of Gramineae, involving 58 species of naturally infected grasses and cereals, indicate that *S. graminum* is not a plurivorous species. A robust spored race of *S. Calamagrostidis* occurs on *Agrostis palustris* (Oregon race 1) (FIG. 4) in Oregon while the filiform-spored species on wheat

is *S. Tritici* (FIG. 5), which is very distinct from *S. graminum* (FIG. 3). It is apparent that the widespread concept of *S. graminum*, which is based on Saccardo's description (**11**), is an unjustifiable grouping of at least three distinct species, namely, *S. graminum* proper, *S. Calamagrostidis*, and *S. Tritici*. The indiscriminate assignment of all filiform-spored species of *Septoria* on Gramineae to *S. graminum* should be discontinued. The diagnostic characters of this group of species are summarized in table 1.

TABLE 1

COMPARISON OF DIAGNOSTIC CHARACTERS OF CERTAIN SPECIES OF *Septoria*

Species	Host	Diameter of pycnidia	Average size of spores		Spore	
			Length	Width	Septation	Form
S. graminum Desm.	*Brachypodium sylvaticum*	70–100	24–45	0.8–1.5	0–2	Filiform
S. Calamagrostidis (Lib.) Sacc.	*Calamagrostis epigeios*	84–180	40–55	0.8–1.1	1–5	Filiform
S. Calamagrostidis (Oregon race 1)	*Agrostis palustris*	60–180	40–60	1.0–2.1	1–3	Filiform
S. Bromi Sacc.	*Bromus* spp.	70–170	45–60	1.2–2	2(1–3)	Scolecosporous to narrowly obclavate
S. Tritici Rob. (Oregon race 1)	*Triticum aestivum*	80–150	40–75	1.8–3	3–7	Scolecosporous
S. Brachypodii Pass.	*Brachypodium sylvaticum*	——	45–55	3.5	Multiseptate	Cylindric

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A NEW SMUT FROM SOUTHERN CHILE

GEORGE L. ZUNDEL

While going through the unfinished manuscripts on the Ustilaginales of the late Dr. G. P. Clinton, the description of a *Ustilago* on *Gunnera magellanica,* written in June 1932, was found. The Clinton specimens were lost and only an envelope with data was found, however, Dr. D. H. Linder has furnished more type material in order to check the Clinton description. Since this description has not been published, it has been deemed best to publish it at this time. Dr. Clinton first worked with this species in 1908 and a description was partly written which was modified in 1932 in the form here presented.

Ustilago Gunnerae G. P. Clinton, sp. nov.

Sori forming conspicuous swellings encircling the petioles or even running into the veines at the base of the leaves; spores when young (and possibly when old) rather firmly agglutinated into indefinite balls or masses situated between the epidermis and a central mass of plant tissues but when wet rather easily separating into individual cells which are reddish-brown, smooth (but often showing evidence of enclosing hyphal threads as whitish attachments), subspherical to broadly elongated and irregular through pressure, 14–18 μ (rarely longer) in length.

On *Gunnera magellanica,* Punta Arenas, Magallanes, Chile, March 2, 1906. R. Thaxter, Coll.

One can not be sure of the genus of this smut since it might be placed by different authors under different genera; however, it does not seem to the writer (G. P. C.) to merit distinction as a new genus. As Thaxter's specimens may be immature and as the germination of the spores are not known, it can be placed under *Ustilago* until more is known about it. There are no signs of sterile fungous cells and the spores are grouped in masses rather than as distinct spore balls. So far as the writer has been

able to learn, no smut has been described on this host or any of the genera of the family, Halorageae, to which it belongs.

G. P. C.
June 1932.

Type material is deposited in the Farlow Herbarium, Harvard University as Accession No. *7760* and also in the Zundel Herbarium.

PENN. STATE COLLEGE

FASCIATION IN THE SPOROPHORES OF CLITOCYBE TABESCENS

ARTHUR S. RHOADS

(WITH 1 FIGURE)

An interesting case of fasciation was observed in one of three well-developed clusters of the toadstools of *Clitocybe tabescens* (Scop.) Bres., on November 10, 1936, following heavy rainfall.

FIG. 1. Fasciation of sporophores of *Clitocybe tabescens*, showing a normal one for comparison.

These clusters occurred at the base of an old rotted oak (*Quercus laurifolia* Michx.) stub in a narrow fringe of hammock forest along the Indian River at Rockledge, Florida. In the abnormal cluster, which contained ten sporophores, three good-sized ones exhibited conspicuous fasciation. The remaining sporophores were normal, except that the stipes of three each showed a slight widening at the point of attachment to the decurrent gills. The fasciated sporophores comprised two exterior ones of the cluster

and one of interior origin, the stipe and pileus of which had grown outward. The stipes of these fasciated sporophores ranged from normal diameter at the base to a width of 4 cm. in two specimens and up to 5.5 cm. in the third (FIG. 1). In the latter case the stipe was bent sharply to one side near the upper limit. The extreme fasciation of the stipes of these three sporophores resulted in a narrow, elongated coxcomb type of pileus. Although the writer has observed this fungus fruiting over a period of several years, this is the first instance where fasciation of the sporophores has been noted.

FLORIDA AGRICULTURAL EXPERIMENT STATION

THE PRESENCE OF ENCRUSTED CYSTIDIA IN THE HYMENIUM OF POLYPORUS ZONALIS

S. R. Bose

(WITH 1 FIGURE)

Polyporus zonalis is a common saprophyte in the eastern tropics, growing on logs, prostrate trunks, stumps, wooden posts, etc.; once it was dug out about six feet below the ground growing on

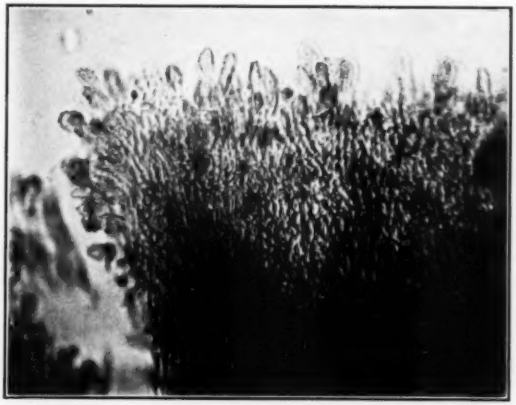

FIG. 1.

the buried leg of a wooden post. It has been reported also from the western tropics (Porto Rico) and from the temperate region of North America (Washington, Texas, Missouri) by Dr. Overholts. It is not found in Europe. *Fomes lignosus* and *Polyporus microporus* are now regarded as its synonyms. In the Trans. Brit. Myc. Soc. (1928) Mr. Petch has entered fully into the history and reconsideration of the synonyms. According to him the so-called "*Fomes lignosus*," which is really an unnamed species, is a parasite and never grows on fallen branches, while *Polyporus zonalis* is a

ubiquitous saprophyte. I have dealt with this point fully in Ann. Myc. in 1937.

Heavily encrusted cystidia (FIG. 1) are found distributed in the basidial layer within the pore tubes of *Polyporus zonalis* examined from various parts of Bengal, Bombay, Ceylon, South Burma (Tenassirim), Andaman Islands, Singapore, Philippine Islands and from the United States—Washington (Dr. J. R. Weir's and Dr. C. J. Humphrey's collections). They are more abundant towards the mouths (the outer margins) of the pore tubes. It has, thus, an important and reliable anatomical feature which has apparently not been noticed by previous workers. In artificial cultures from spores and tissues, these cystidia become very prominent on account of very heavy encrustation giving rise to a distinctly warty appearance all over.

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ON A NEW RAVENELIA FROM INDIA

B. B. Mundkür and N. Prasad

(with 1 figure)

A rust which formed amphigenous and caulicolous sori on an *Acacia,* subsequently identified as *Acacia modesta* Wall., was collected on January 6, 1937, at Delhi. When the pinules were examined with a hand lens minute, nearly circular, deeply dark-brown structures which looked black in mass were distinguished on the ventral side. These were clusters of sori which were also found on the petioles and the branches. Well defined spots were not manifest but near the sori the leaf was pale-yellow with well defined necrotic areas reminiscent of similar zones produced by the stem-rust on certain varieties of wheat. The sori were 0.5 to 1.00 mm. in size, slightly longish, becoming confluent on rupturing.

Microscopic examination of the rust indicated that it belonged to the genus *Ravenelia.* Pycnia, aecia and uredia were not present in the collections made on two different occasions, only telia being found in many of the preparations. The teliospores were fascicled into compact heads which were convex, hemispherical to orbicular, rarely oval with an alantoid appearance in side view (fig. 1). They were chestnut-brown in color, smooth and subepidermal in origin. The telial heads measured (200 measurements) from 83 to 128 μ (chiefly 105 μ), in diameter. They were borne on short, deciduous, hyaline stalks which were composed of numerous hyphae. Paraphyses were present in the sori.

In each telial head there were 10 to 12 cells across, on every diameter, all the cells being uni-cellular. The wall of the central cells was 5–7 μ thick at the apex and it was noted that the coloring matter was chiefly confined to this upper layer, the lower portion of the cells being yellowish to hyaline. Individual cells measured 18–22 $\times$ 8–11 μ.

The base of the heads was encircled by hyaline, oblong-ovate, pendent cysts which swelled and diffused in liquid media. Their number was equal to that of the number of teliospores in the head.

The genus *Ravenelia* has been studied in detail by Dietel (1894 and 1906) and Long (1903). Long divided it into three separate genera on the basis of the number of cells in the teliospores and on the presence or absence of a pseudo-peridium in the aecium.

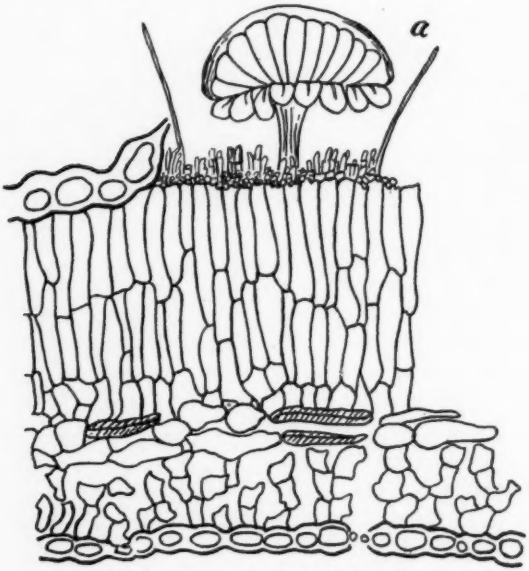

FIG. 1. *Ravenelia Taslimii* Mundkur.

Sydow (1921) went a step further and segregated the genus into eight genera on the basis of the number of cells in the individual teliospores and on the presence or absence of the different fruiting-stages. Doidge (1926) and Arthur (1934) have adopted a more conservative view and the former subdivides the genus into two subsections: *Haploravenelia* in which teliospores have a single cell and *Pleoravenelia* in which they have two cells each. The specimen under study falls into the subsection *Haploravenelia.*

Twenty-six species of *Ravenelia* have been recorded on the genus *Acacia* though none have so far been reported to occur on *Acacia modesta.* Of these only six species belong to the subsection *Haploravenelia* and have cysts equal in number to the number of teliospores in a head. A comparative statement of

the characteristics of these six species and the species under study is given in Table I.

TABLE I

CHARACTERISTICS OF SOME SPECIES OF RAVENELIA

Name	Habit	Stages present	Cells in a head, across	Size of heads	Size of teliospores
R. inornata Diet.	Epiphyllous, petiolicolous	I, III	8–12	115–175 μ	31–60×12–18 μ
R. natalensis Syd. et Evans	Caulicolous	I, II, III	3–12	30– 50 μ	20–27×13–17 μ
R. Peglerae Doidge.....	Amphigenous, caulicolous	III	6–8	60–110 μ	27–40×10–15 μ
R. australis Diet. et Neg..	Epiphyllous	II, III	9–10	70–125 μ	25–40×12–15 μ
R. Thornberiana Long.....	Amphigenous, caulicolous, fructicolous	II, III	4–5	70– 90 μ	——
R. Stevensii Arth.....	Hypophyllous	II, III	3–6	40– 63 μ	6–19 μ long
Ravenelia sp....	Amphigenous, caulicolous	III	10–12	83–128 μ	18–22× 8–11 μ

A critical examination of the species whose measurements are recorded in the table indicates that the *Ravenelia* sp., under study differs from the others in several respects. The species nearest to it is *R. australis* but this is an epiphyllous rust. It has a uredial stage which the species under study does not presumably have and its teliospores are larger both in length and breadth, possessing a smaller number of cells across in the telial head. The species on *Acacia modesta* is therefore considered new and the name *Ravenelia Taslimii* after the collector is proposed for it.

Ravenelia Taslimii Mundkür, sp. nov.

Pycnia, aecia and uredia wanting. Telia amphigenous, caulicolous, dark, irregularly in clusters, confluent, 0.5–1.0 mm. in size, subepidermal. Paraphyses present. Pale-yellow necrotic areas on pinnules. Telial heads convex, hemispherical to orbicular, rarely oval, alantoid in side-view, smooth, chestnut-brown, 83–128 μ, chiefly 105 μ, in diameter, on short, thick, hyaline, deciduous stalks. Spores 10–12 × 8–11 μ with 5–7 μ thick epispore at apex. Cysts as many as individual teliospores, hyaline, oblong-ovate, pendent, swelling and diffusing in water.

Pycnidiis, acidiis atque uredosporiis carens. Teleutosporis amphigenis, fuscis irregulariter in turmas confluentibus, 0.5–1.0 mm. extensas, sub. epidermicas. Paraphysibus praedita. Areis pallidis glaucis necroticis super pinnulas. Teleutocapitibus convexis, forma hemisphaerica ad orbicularem, raro ovalem; alantoide obliquo, terso, brunneo, 83–128 μ, praecipue 105 μ, in diametro, super pedunculos breves, crassos, hyalinos, deciduos; sporae 10–12 × 8–11 μ, cum epispora 5–7 μ lata ad apicem. Cystibus pari numero atque individuis teleutosporis, hyalinis, oblongo-ovatis, pendulis, inflatis, et in aqua diffluentibus.

Super *Acaciam modestam* Wall. In loco dicto Ridge, New Delhi. 6 Jan et 20 Febr. anno 1937.

On *Acacia modesta* Wall. on Ridge, New Delhi, India. Jan. 6 and Feb. 26, 1937. Collected by Mr. Mohammed Taslim and N. Prasad.

Type specimens deposited in the herbarium of the Imperial Agricultural Research Institute, New Delhi, Imperial Mycological Institute, Kew, Kew Herbarium, Herbarium of the New York Botanical Garden and the Farlow Herbarium.

SUMMARY

A species of *Ravenelia* on *Acacia modesta* Wall., has been studied in detail and compared with other species occurring on the genus *Acacia*. The species has been given the name *Ravenelia Taslimii* Mundkür.

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AN EARLY OCCURRENCE OF TAPHRINA SACCHARI IN WISCONSIN[1]

ANNA E. JENKINS

(WITH 1 FIGURE)

The existence of a specimen of *Taphrina Sacchari* Jenkins (**8**) on sugar maple (*Acer Saccharum* Marsh.) from Wisconsin, collected at Madison, has recently been discovered. The specimen bears the locality and date, "Drive, June 1904," and was labelled and filed in the Herbarium of the University of Wisconsin, as *Gloeosporium saccharinum* Ellis and Ev. The location of the drive referred to is on the south side of Lake Mendota, west of the campus. The specimen thus antedates the curatorship of the Herbarium by Dr. J. J. Davis, which began in 1911 (**1**), and there is no evidence that the specimen ever came to the attention of this authority on the parasitic fungus flora of the state.

The published enumerations of this flora as assembled by Davis, include an earlier list by Trelease (**10**), and thus embrace the period from 1884 until 1937 (**4**), when the last list was published. Comparatively few species (less than 10) are represented on sugar maple and there is no mention of the specimen from Madison, or of a species of *Taphrina* on maple. His own collections of *Gloeosporium saccharinum* from Racine and Waukesha are referred to in his original list, published in 1893 (**2**). The type specimen of this species is, of course, his own collection from Racine, August 1890 (**5**), represented in Ellis and Everhart's North American Flora *2668* (**6**). This specimen, as well as others of this species collected elsewhere in Wisconsin, by Davis, and filed in the Herbarium of the University, including his separate private collection, exhibit leaf necrosis distinct from that produced by *T. Sacchari*.

[1] The observations reported in this article were made during July and August, 1938, while the writer was completing certain taxonomic research on the North American species of *Taphrina* on maple (*Acer*), and through the courtesy of E. M. Gilbert, was working in the Botany Department of the University of Wisconsin.

On the other hand, the leaf spot or blister of the leaves from Madison (FIG. 1) is of identical appearance with the two specimens of *Taphrina Sacchari* in the Herbarium. These are labelled "*Taphrina* sp." as sent to Doctor Davis by the writer during her early investigation of this species. Of these two, the one from Maine bears the same data as the type specimen of this species on sugar maple, except that it was collected later during June 1922; the other is from the locality in New York where the fungus is

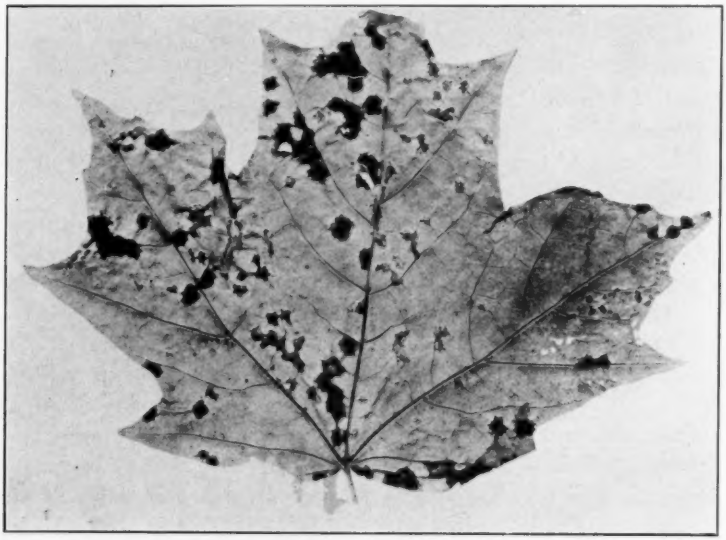

FIG. 1. *Taphrina Sacchari* on lower leaf surface of sugar maple, Madison, Wis., June 1904. Photograph by Eugene Herrling.

known to have been present since 1922 (**8**), and was collected by the writer in August 1938. The asci and ascospores on the June collected specimen from Madison are mature as illustrated elsewhere (**9**), and there seems to be no question of their correspondence with those of *T. Sacchari* as previously studied. On the basis of this identification the specimen from Madison was included among the specimens of *Taphrina Sacchari* examined as cited in connection with the original description (**8**).

The specimen is of special significance as the earliest mycological collection of this fungus yet known, and as the first and only record from Wisconsin. The *Taphrina* was, of course, discovered only in 1922, following the apparent epiphytotic outbreak of the disease it causes, and the first available record is July 7, 1894, based on the presence of lesions on a phanerogamic specimen collected at Lansing, Mich. (**7**, **8**). Part of the collection of the specimen from Madison will be filed in the Mycological Collections of the Bureau of Plant Industry, under the accession number 72882.

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WASHINGTON, D. C.

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NOTES AND BRIEF ARTICLES

Mycology in the Encyclopaedia Britannica

On page 114 of this volume of *Mycologia* I dwelt on the scantiness of the information on Mycology contained in the popular encyclopedias. The Britannica in each of its editions from the 9th to the 13th, the latest of which I had knowledge at the time, was one of the seven sets mentioned. I am indebted to Dr. D. B. Gilchrist, Librarian of the Rush Rhees Library, University of Rochester, for calling my attention to the mycological topics treated in the 14th edition. After examining this edition an acknowledgment of the facts seems warranted as well as a statement of the belief that mycologists who have not seen it should know the titles treated there in the encyclopedic manner by competent writers.

In this, the 14th edition, " Mycology " is in alphabetic place and is defined in two lines as the science of Fungi. The reader is then referred to the article " Fungi " written by Prof. R. J. Tabor, B.Sc., Imperial College of Science, South Kensington. Condensed into 27 columns, he briefly but evenly reviews the science of mycology. He estimates that there are about 100,000 species of fungi and that notwithstanding their extremely evanescent nature some beautiful fungus fossils have been found in time as remote as the Devonian period.

A number of important groups are treated in new articles to be found in their respective alphabetic places, e.g. Mushrooms, Puff-balls, Truffles, Yeasts, Parasitic Fungi in Plant Pathology, Smuts, Lichens, Mycorhizae and Morels. Plant Pathology by Prof. Wm. Brown covers 17 columns; Lichens by Miss A. Lorraine Smith, 11 columns well illustrated; etc.—John Dearness.

Fungi of the Human Ear

During the writer's early days at The New York Botanical Garden, an associate who had been suffering with a severe infec-

tion of the ear came into the laboratory with a statement that he believed there was a fungus on the cotton plug which had just been removed from his ear. A portion of this material was placed under the microscope and revealed beautiful heads of the fungus *Aspergillus,* apparently *Aspergillus nigricans.*

The victim had suffered severe agony from this infection for years previous, and in spite of repeated efforts it had failed to respond to medical treatment. Having learned what the fungus was, he himself diagnosed his own disease and learned the remedy. The following note by Dr. A. B. Stout was published in the Journal of The New York Botanical Garden (**13**: 126. 1912.):

"The disease known as mycosis of the external ear of man is not uncommon. Cooke describes as a new species, *Aspergillus nigricans,* which had been obtained from the human ear. Later he again describes and also gives figures of this mould.

General descriptions of cases of mycosis of the external ear have appeared in various medical journals and books. One of the more recent of these is by the noted specialist Ballenger, whose discussion may be here summarized as follows: The fungus forms a membrane black or grayish in color and velvety in texture which covers the osseous portions of the canal, although the drum head and cartilaginous portions of the canal may also be covered. If the epidermis alone is affected there may be no symptoms. If the true skin is attacked there is swelling and inflammation with pains, itching and deafness. The mycelium may extend to the middle ear or even to the mastoid cells.

The source of the infection is unknown. It is noted, however, that the disease is quite common among bakers and among the poor who are living in unsanitary conditions. It is stated that various species of fungi have been found growing in the ear, but the most common species are *Aspergillus niger, A. flavus* and *A. fumigatus.*

In the treatment, a long list of antiseptic mixtures and powders have been used without general success. In fact, the fungus appears to thrive in spite of treatment with the ordinary solutions of carbolic acid, boric acid and mercury bichloride. Alcohol is, however, an effective remedy, and when dropped in the ear once or twice daily for about four days it effects a complete cure.

A case of infection of the ear by *Aspergillus nigricans* Cooke has recently been brought to the attention of the writer. In this case there has been also repeated infections with Micrococcus, resulting in small abscesses. Several physicians and ear specialists consulted from time to time were led by this condition to overlook the presence of the fungus which was evidently of primary importance. The treatment with mercury bichloride (1:1,000) checked the infections due to the micrococci, but the fungus continued to develop, at times almost filling the ear cavity with mycelium and producing

an abundance of spores. In this condition it was easily isolated in pure cultures. At present report the treatment with alcohol appears to have ertirely removed the infection from the ear."

In lecturing on the fungi the writer has had occasion to refer to this incident many times, and recently took the opportunity to check up and found that the remedy [1] indicated above has been entirely successful in suppressing this disease. In response to the publication of the note quoted above, numerous inquiries have come in and it has been found that this disease is much more prevalent than one might suspect, especially in tropical countries. For this reason it is thought that this information might be of interest to the readers of Mycologia.—FRED J. SEAVER.

[1] The patient writes as follows: "50% alcohol kills the fungi—but tends to irritate membranes—so I also use an ointment obtained from an ear specialist—use this on cotton swab in removing wax—about twice a month. By using alcohol when I suspect fungi may be present—and the ointment more frequently—have had no micrococci infections for several years—only one or two since treatment began.

INDEX TO VOLUME XXX[1]

INDEX TO AUTHORS AND TITLES

[1] This index was prepared by Gussie Miller.

INDEX TO GENERA AND SPECIES

New names and the final members of new combinations are in **bold face type**. Mistakes in the spelling of scientific names encountered in text are corrected in the index.